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IMPEDANCE CHANGES IN FROG'S MUSCLE ASSOCIATED WITH ELECTROTONIC AND "ENDPLATE" POTENTIALS

BERNHARD KATZ
Kanematsu Memorial Institute of Pathology
Sydney Hospital, Sydney

(Received for publication September 3, 1941)

INTRODUCTION

IN A previous paper (14) it has been shown that a motor nerve impulse, on its arrival at a blocked myoneural junction, gives rise to a brief subliminal depolarization of the muscle fibre which dies out in the vicinity of the endplate within about 20 msec. This "endplate potential" (e.p.p.) resembles in many of its properties a subthreshold catelectrotonic potential change. In the present paper a further analogy is provided by the diminution of impedance found under both conditions.

The occurrence of a small impedance loss at the *cathode* preceding and possibly initiating the much more drastic propagated change (4, 11), bears an interesting relation to the problem of excitation, the setting up of local responses and their transition into "all-or-nothing" impulses.

The impedance loss during the e.p.p. differs in its initial time course from that accompanying a similar catelectrotonic potential; a comparison of the two effects suggests that, at the neuromuscular junction, a nerve volley may produce an initial membrane change which rises and falls more rapidly than the e.p.p., and this is discussed in relation to the transmitter problem.

METHODS

General technique of impedance measurement

Changes of electric potential and of transverse impedance were measured on the frog's sartorius which was isolated together with its nerve. For impedance measurements, platinized platinum electrodes of 1 or 0.5 mm. width were applied to the muscle transversely. Muscle and electrodes formed one arm of a Wheatstone bridge and were balanced by a combination of variable resistance and capacity in parallel.

A resistance-capacity tuned oscillator as described by Terman *et al.* (23) was used providing sine waves of very low harmonic content. The bridge output was connected to a resistance-capacity coupled amplifier and cathode ray oscillograph. Impedance changes of less than 0.01 per cent could be measured. The response of the amplifier to an instantaneous alteration of bridge balance was complete within less than 0.3 msec.

The 2 Pt wires were perpendicular to the muscle, but because chance asymmetries were found to act as a differential lead for action potentials, recording a potential of reduced size (10–15 per cent) and quickened time course whenever a spike or e.p.p. was set up (7). To avoid its interference with the recorded bridge output, a high-pass filter cutting off frequencies below 2000 c.p.s. was inserted between bridge and amplifier.

The bridge input consisted of alternating current (A.C.) at 5000 c.p.s., its strength being well below half-threshold, probably no more than 10 per cent of the direct muscle threshold. Since this work is concerned only with the time course of the impedance changes, observations at several frequencies were not required. 5000 c.p.s. was a frequency high enough to map out the time course of events during spike and e.p.p., yet low enough for adequate recording of membrane changes (5, 16). The A.C. strength was so low that it did not add appreciably to the excitatory effect of the e.p.p., even if the latter was only slightly

below threshold. Furthermore, halving or doubling the strength of the A.C. merely changed the bridge sensitivity without altering the impedance changes. Thus, the measuring current did not seem to affect the condition of the tissue.

In the following, impedance $Z = R/\sqrt{1+R^2\omega^2C^2}$, phase tangent $\tan \phi = R\omega C$, R and C being the adjustable parallel resistance and capacity respectively, and $\omega = 2\pi \times$frequency ($=31{,}000$/sec.). $R^2\omega^2C^2$ was always less than 0.2, R about 2000 to 3000 Ω.

During spike, catelectrotonic potential, and e.p.p. a transient diminution of impedance and phase tangent occurs, but while the changes during the spike are of the order of 10 per cent, those during the e.p.p. are only about 0.1 per cent. The most accurate way of tracing their time course would be to unbalance impedance and phase angle at rest and find, by trial and error, such values of R and C as would give balance at various moments during the change. However, as Cole and Curtis (7) found, the result of this method does not differ appreciably from the time course of the bridge record itself, obtained with balance at rest or during the maximum change (which implies that the change of complex impedance, in a resistance *vs.* reactance diagram (5), follows approximately along a straight

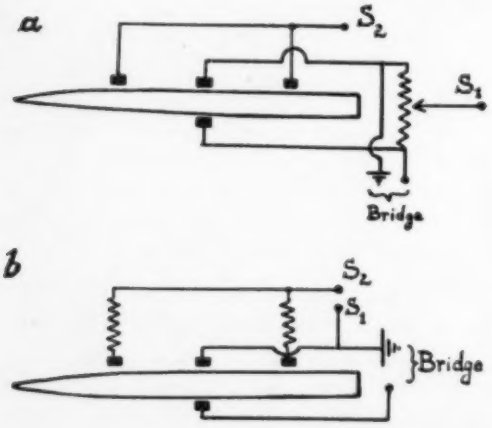

FIG. 1. Sartorious muscle with electrodes for direct stimulation and impedance measurements. *a* and *b*: 2 alternative circuits (see text). S_1, S_2: Stimulating electrodes.

line). Now, with changes of only 0.1 per cent, the method of balancing at various moments becomes impracticable, partly because of residual harmonic content and partly because of slow drift of the resting impedance. For the same reasons, it was inconvenient, and often impossible, to work with sharp balance at rest or at the peak. The procedure usually adopted was to set the bridge slightly off balance and to record the amplitude changes on the oscillograph. A band of oscillations was recorded (*e.g.* Fig. 9) which during the e.p.p. increased or decreased in width, depending upon whether the impedance of the variable bridge arm had been made greater or smaller than that of the resting muscle. In this way, the time course of the impedance change could be obtained with sufficient accuracy, provided that the direction of the complex impedance unbalance, at rest, was in phase with that of the impedance change (*i.e.* coinciding with the direction of the mentioned straight line in the resistance-reactance diagram). This direction was found by adjusting the variable impedance and phase angle so as to get (i) a maximum change of band width; (ii) an opposite, but symmetrical change of band width when the sense of the resting unbalance is reversed; (iii) a minimum phase shift of unbalanced fundamental frequency (as indicated by its relation to the unbalanced harmonic content). In most experiments these conditions were obtained by unbalancing the parallel resistance R, while leaving the capacity C at balance.

The measurement of the records provided no difficulty in spite of the high amplification and consequent unsteadiness of the baseline (overall voltage amplification of bridge output was about 10^6). A more serious distortion was due to the cathode ray tube, the vertical sensitivity of which changed somewhat at different positions of the horizontal sweep; but this could easily be corrected for.

E.p.p.'s and muscle spikes, due to nerve stimulation, were recorded by using the im-

pedance electrodes connected together as the "active" lead and a Pt electrode on the end of the muscle as reference lead.

Impedance measurements during direct stimulation

As direct stimulus a double condenser discharge was used rising to a peak in 0.8 msec. and falling to one-half in about 2 msec. This particular type of stimulus was chosen because it would set up an electrotonic potential similar in shape to the endplate potential.

The muscle was deeply curarized (curarine concentration of 9 μmol. per l.) so that any changes, *e.g.* endplate potentials, due to excitation of intramuscular nerves would be quite negligible. The stimulus was delivered, and synchronized with the time base, by a Lucas pendulum. The current was led into the muscle in two different ways (Fig. 1), entering either at one or both impedance electrodes and leaving at two other Pt electrodes about 1 cm. away on either side. By using both impedance electrodes as a common stimulating lead, connected together by a high resistance (Fig. 1*a*), potential changes due to stimulus artifact could be minimized. On the other hand, with the arrangement of Fig. 1*b*, it was possible to earth the stimulating apparatus and so to avoid baseline disturbance during the fall of the pendulum. The results were the same with either method. To make the time course of the stimulus independent of electrode polarization, a resistance of at least 50,000 Ω was placed in series with the muscle. Oscillograph records showed that the shape of the current pulse was unaltered when preparation and electrodes were short-circuited.

If the muscle was replaced by a saline soaked wick, no detectable impedance change (*i.e.* less than 0.001 per cent) was produced by currents somewhat stronger than employed in the experiments.

Results

A. *Impedance Changes Elicited by Subthreshold Electric Currents*

At the outset of this work, there were reasons to believe that a subthreshold electric current would not alter the muscle impedance at all. Cole and Curtis (7) had been unable to find an impedance change during the subthreshold "foot" which precedes the propagated action potential, and their work suggested that a change of membrane permeability, and an associated impedance change, would occur only after attainment of a critical depolarization. The present experiments, however, in agreement with Dubuisson (11) and Cole and Baker (4) show that this view must be modified. As illustrated in Fig. 2, any subthreshold current pulse produces an increase of muscle impedance at the anode, and a somewhat greater decrease at the cathode.

The size of the subthreshold impedance changes is relatively small, *e.g.* at threshold for the most excitable fibres the cathodal impedance loss amounts to about 0.5 per cent, which compares with a diminution of about 10–15 per cent during a maximal, fairly synchronous, muscle spike (Section II A).

The time courses of the impedance change is shown in Fig. 3. Its relation to the applied current pulse is of particular interest. The impedance change rises gradually and reaches a maximum during the declining phase of the stimulus. It then falls slowly, in an approximately exponential fashion, decaying to one-half in about 7–8 msec. This time relationship is similar to that of the electrotonic potential of muscle (Section I B), cf. also Schaefer, Schölmerich and Haass (22), and the rate of decay is about the same as that of the e.p.p. (14).

In normal muscle, the time course was approximately the same for all anodal and subthreshold cathodal stimuli, though often the decay was found to be slightly quicker at the cathode than at the anode. If threshold was exceeded, propagated impulses occurred, accompanied by a quick transient impedance loss and followed, after several milliseconds, by a change associated with the contraction (see below). It is interesting to note that, at

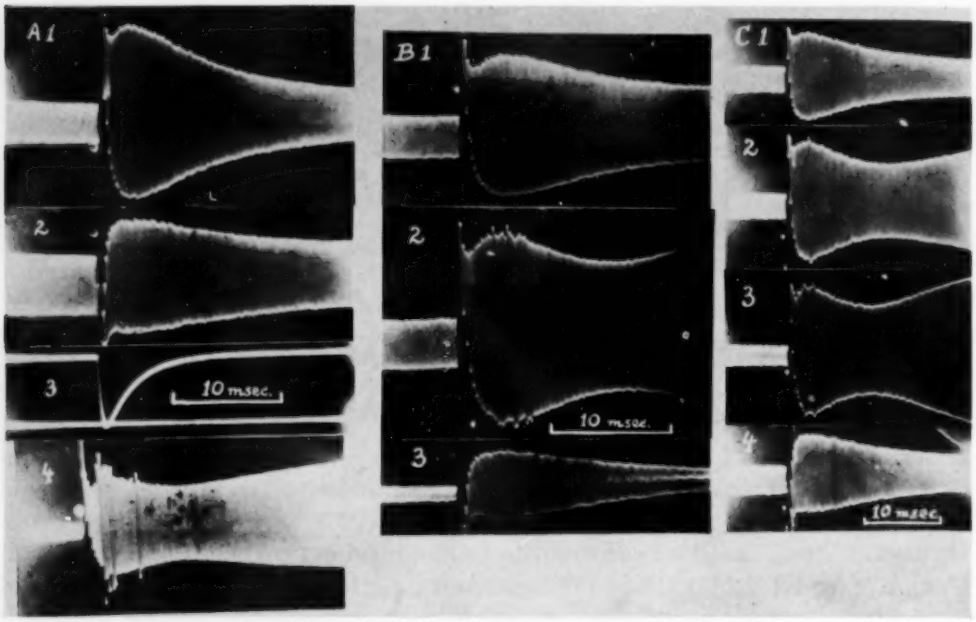

FIG. 2. Impedance changes during direct stimulation. Bridge unbalance, at rest, was of opposite sense at anode and cathode so as to give always an increase of band width. Circuit of Fig. 1*b* was used (hence stimulus artefact not balanced). *A:* 24°C. *1:* cathode, 14 V., impedance drop 0.4 per cent; *2:* anode, 14 V., impedance rise 0.16 per cent; *3:* record of stimulus; *4:* cathode, superthreshold stimulus (lower amplification), note spikes and later change due to contraction. *B:* 22.5°C. *1:* cathode, 33 V., imp. drop 0.29 per cent; *2:* cathode, 38 V., imp. drop 0.41 per cent, spikes and contraction change; *3:* anode, 33 V., imp. rise 0.16 per cent. *C:* 24°C. *1–3:* cathode; *1:* 12.5 V., imp. drop. 0.255 per cent; *2:* 17 V., threshold current, note little spike on top of imp. change (−0.385 per cent) and later contraction change; *3:* 19 V. additional spike and contraction change. *4:* anode, 17 V., imp. rise 0.205 per cent.

threshold, the propagated disturbance takes off at the peak of the catelectrotonic impedance loss. With still greater current intensities the latency of the propagated change diminishes and its size increases, more fibres being involved. This again confirms the general similarity of potential and impedance change (22).

The impedance change during contraction has been studied in detail by Bozler (2) and Dubuisson (12) who have shown that, unless the muscle is fixed rigidly, the observed change is largely due to movement and local thickening. In the present work, no special attention was paid to the contraction change except that it served as a sensitive subsidiary

threshold index. It was absent with subliminal direct stimulation, or with nerve stimulation in completely curarized muscle (Section IIB). It became appreciable only after the spike and its accompanying impedance change had declined to a small remainder. The separation between the impedance changes, accompanying spike and contraction respectively, was largest when recording from the cathode or, in the case of nerve stimulation, from the junctional region, that is before any conduction along the muscle has occurred.

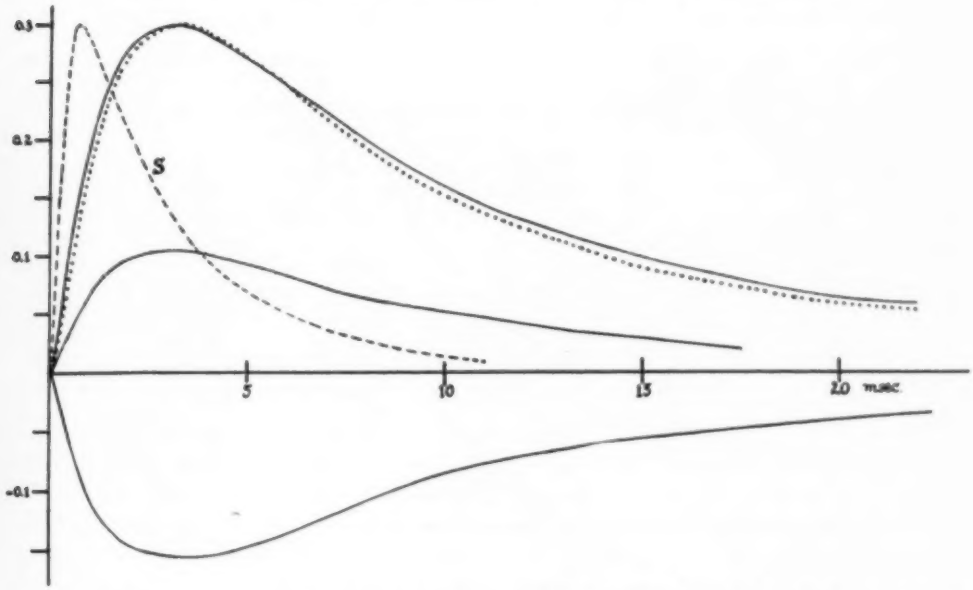

FIG. 3. Time course of impedance changes (full lines) and stimulus S (broken line). 24°C. Ordinates: impedance decrease (upwards) and increase (downwards) in p.c. Current strength, in p.c. of threshold for most excitable fibres, successively from above: 0.78, 0.38; −1.0 (anodal). The *dotted curve* shows the approximate time course of the electrotonic potential (see Section IB).

If the muscle was damaged, *e.g.* by application of a prolonged polarizing current, the threshold increased progressively, while the subliminal impedance changes diminished in

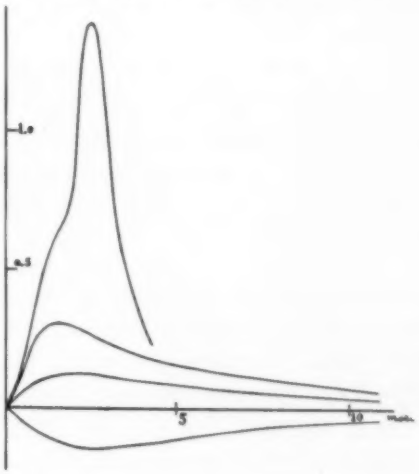

FIG. 4. Impedance changes in injured muscle (see text). Ordinates: per cent impedance loss. Cathodal changes upwards, anodal downwards. Current strength in relative units, successively from above: 1.0 (subthreshold), note spike, 0.73 (below threshold), 0.52; −1.0 (anodal).

size and quickened in their time course. At the cathode, with currents of slightly less than threshold strength, a relatively large quick component of the impedance loss was observed (Fig. 4), possibly the counterpart of a non-propagated response. This effect, though only found in deteriorating muscle, is of some interest, since it may be related to the marked local responses (20, 21; unpublished experiments by Kuffler on single muscle fibres) which have been observed at the cathode under similar conditions.

The relation between applied current strength and impedance change was always non-linear. In Fig. 5 the two variables are plotted, and a character-

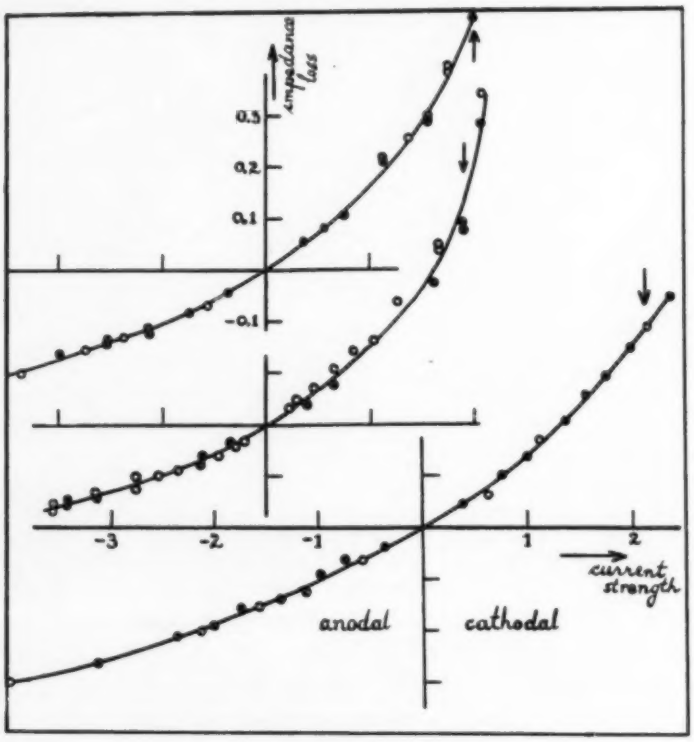

FIG. 5. Relation between impedance change and current strength. Three different experiments. Ordinates as in Fig. 3 (marked at every 0.1 per cent change). Abscissae: Current strength in relative units. (Cathodal currents and impedance loss are represented as positive.) Appearance of spikes marked by arrow. Hollow circles: series of observations with increasing current; full circles: reverse series.

istic curve is shown the slope of which increases continuously at the cathode as the current intensity approaches threshold, while it decreases with increasing anodal current strength. This type of curve may be of considerable importance; however it cannot be interpreted accurately until more is known about the current distribution in the muscle and the absolute values of membrane resistance and capacity.

B. *The Electrotonic Potential of Muscle*

It was desirable to compare the time course of the sub-threshold impedance changes with that of the electrotonic potential. This could not be directly recorded, at the point of stimulation, because the double-condenser pulse introduced a large artifact which could not be balanced without

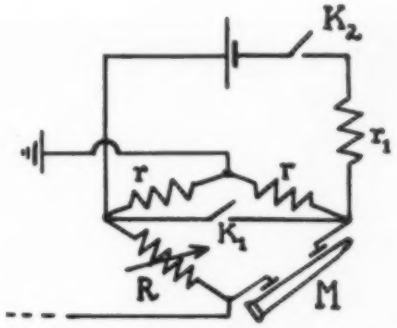

FIG. 6. Circuit for determining time course of polarization potential of muscle. K_1, K_2: break contacts of Lucas pendulum. $r_1 = 0.1$ MΩ. $r = 1000$ Ω. *R:* balancing resistance. *M:* sartorius muscle.

distorting the electrotonic potential to an unknown degree. Its approximate time course was then found in the following way. A weak constant current (5–10 per cent of threshold strength, lasting about 60 msec.) was applied to the muscle by means of 2 non-polarizable electrodes (chlorided silver plates in 2 pools of Ringer which were bridged by 10–15 mm. length of muscle; the muscle was connected with the saline pools directly or by 2 bridges of soaked wood, 0.5 mm. thick, so as to make the contact area similar to that in the previous experiments).

The development of the polarization potential at the muscle membranes was recorded as shown in Fig. 6. The preparation was inserted into a direct current bridge, fed by the constant current pulse and balanced at various moments of the current flow, *e.g.* at the beginning or end (Fig. 7). The bridge output gives us then the time course of the back e.m.f. developed by muscle and electrodes. Controls showed that neither electrode polarization nor amplifier distortion were appreciable. (i) If the muscle was replaced by a saline wick, the polarization potential was reduced to less than 2.5 per cent (Fig. 7*c* and *d*). (ii) The amplifier response to a rectangular voltage input reached 90 per cent in about 0.1 msec., and declined to one half in about 0.7 sec. Thus, potentials lasting 60 msec. would be slightly distorted (Fig. 7*a*). This error, however, can be eliminated by adjusting the bridge for balance during the steady state of current flow (Fig. 7*b*).

Thus, the records give the time course of the polarization potential of the muscle itself (*i.e.* of its electrotonic potential). It is approximately exponential, the average "half-time" (*i.e.* time for 50 per cent decay or rise) being about 7 msec. (actually, successive half-times become progressively larger: in the example of Fig. 7, 5.6–7.7–7.9–8 msec.). This is nearly the same as the average time for half-decay of the endplate potential (14).

From these records (Fig. 7) one can determine, by graphical analysis, the approximate time course of the electrotonic potential for any shape of current pulse, *e.g.* for the double-condenser-discharge used above. The analysis is based on the fact that any electric stimulus may be treated approximately as a series of brief constant current pulses, the total electrotonic potential being made up by their summed contributions. The result of such an analysis has been plotted in Fig. 3, for comparison with the impedance changes. Rising period and rate of decay are nearly the same as in the impedance records. It is not possible to say whether there is exact agreement between impedance and potential curves, since the determination of the electrotonic potential is approximate, and the data were obtained from different muscles where a 20–30 per cent variation in time scale was usual. A recent report by Cole (3) indicates that, in the squid giant axon, the impedance change lags somewhat behind the potential. This possibility has not been excluded for the frog's sartorius, but as a first approximation the time courses of subthreshold impedance and potential changes in frog's muscle may be taken as identical.

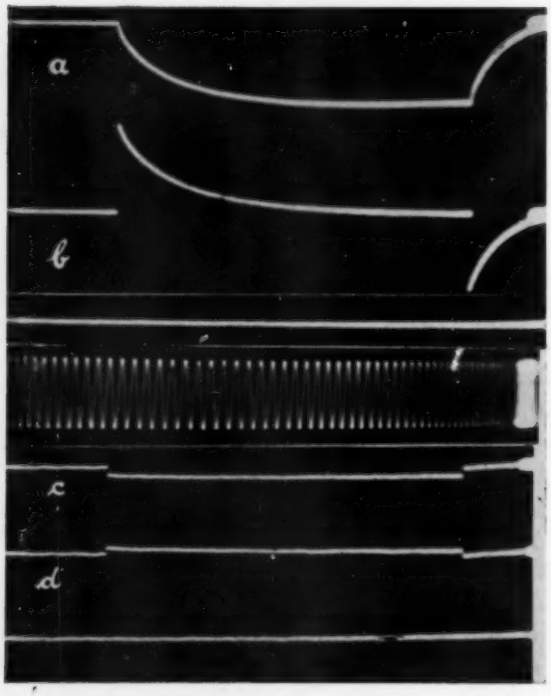

FIG. 7. Polarization potential of muscle during constant current flow. 15°C. *a:* bridge balanced at beginning of current pulse ($R = 9310$ Ω); *b:* bridge balanced during steady state ($R = 11410$ Ω). *c* and *d:* muscle replaced by saline wick. Bridge slightly unbalanced, in opposite sense at *c* and *d* ($R = 2670$ and 2570 Ω respectively). The polarization in *c* and *d* is equivalent to a resistance change, during current flow, of less than 40 Ω, *i.e.* 2 per cent of that in *a* and *b*. Time signal 500 c.p.s.

II. IMPEDANCE CHANGES AT THE NEURO-MUSCULAR JUNCTION

The main object of these experiments was to examine further the nature of the local change which a nerve volley sets up in a completely curarized

muscle. Potential and impedance changes were recorded, before and after curarization, at a junctional region of the frog's sartorius (*i.e.* a region of minimum spike latency, or maximum e.p.p.; see 19).

When comparing impedance and potential records, one must take into account that the two changes are recorded effectively at different points, the former being led off from the whole region between the transverse *Pt*-electrodes (see Methods), the latter only from the edge of the *Pt* electrode which faces the reference lead. However, owing to the scatter of individual endplates, the junctional regions of most muscles extend over an area larger than that of the *Pt* electrodes. Within these regions, movement of the electrodes causes no appreciable change in latency or shape of the potential records and so it is safe to compare them directly with the impedance changes recorded from the centre of a junctional region.

A. *Impedance Changes During the Muscle Spike*

In a non-curarized nerve-sartorius preparation, muscle spike and associated impedance change, due to a maximum nerve volley, was recorded

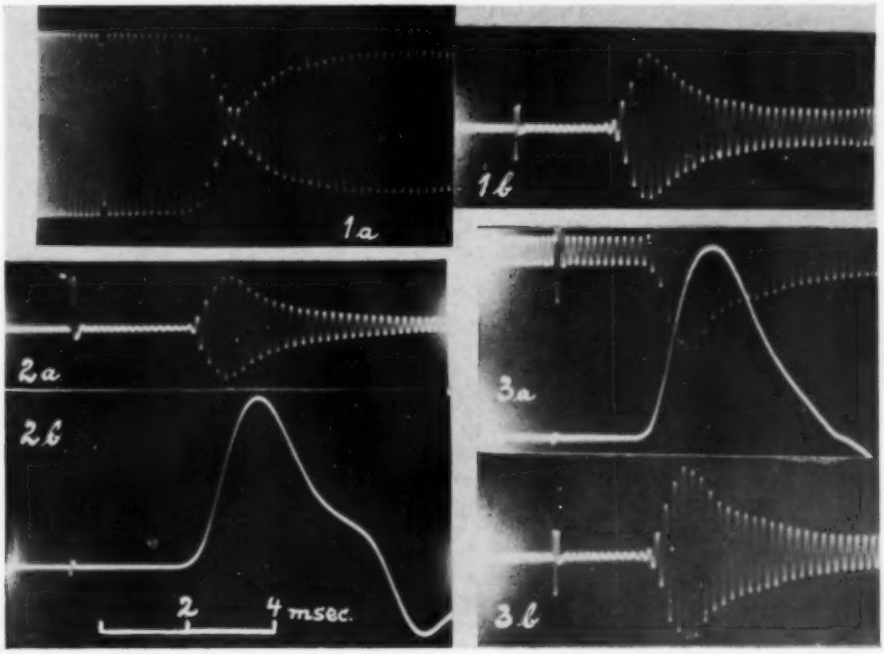

FIG. 8. Action potentials and impedance changes recorded at junctional region of normal sartorius. Maximal nerve stimulus. 1*a* and *b*: 21°C. 8 per cent. Impedance drop. 2 different adjustments of bridge. 2*a* and *b*: 16°C. 6.3 per cent impedance drop; spike 59 mV. 3*a* and *b*: 21°C. 10 per cent impedance drop; spike 63 mV. Note: the last 2 msec. of the impedance record are affected by the beginning of a later change due to the contraction.

from a junctional region (Fig. 8). There was a diminution of impedance and phase tangent of about 10 per cent (mean of 8 experiments: 9 per cent, varying between 5 and 15 per cent). The records are similar to those obtained by Cole and Curtis (6, 7) on Nitella and single nerve fibres, and by Auger

and Fessard (1) on the electric organ, except that the spike effect is followed, after an interval of several milliseconds, by a second impedance change accompanying the contraction (see Section I; cf. 10, 12). Potential and initial

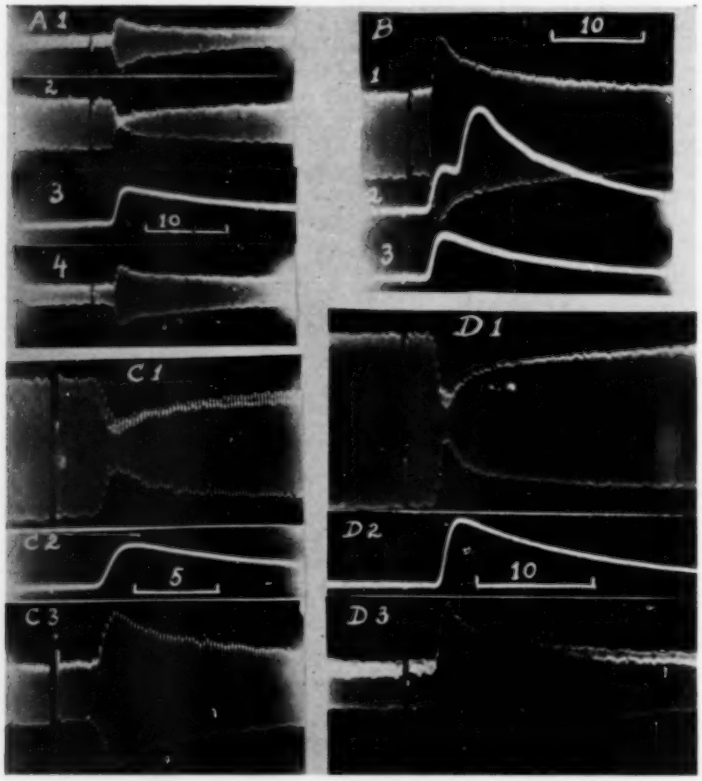

FIG. 9. E.p.p.'s and impedance changes in fully curarized muscle, both recorded from junctional region. Ringer contains five-fold excess of calcium and about 4 μmol. curarine. *A*: 17°C. 0.08 per cent impedance drop (3 different bridge adjustments); e.p.p. 2.7 mV. *B*. 21°C. 0.15 per cent impedance drop, during single e.p.p. (3.4 mV). (Note appearance of small propagating spike on top of second e.p.p.; threshold height of e.p.p. about 7.5 mV). *C*. Faster recording speed. Impedance drop 0.135 per cent; e.p.p. 3.1 mV. *D*. 18°C. Impedance drop 0.125 per cent; e.p.p. 4 mV. Time marks: for *A*, *B*, *D*, 10 msec.; for *C*, 5 msec.

impedance changes begin at about the same time, but the impedance change reaches its peak more quickly, the rising phases, at 22°C., being 1.0 msec. (0.8–1.23 msec.) and 1.4 msec. (1–1.8 msec.) for impedance loss and spike potential respectively.

B. *Impedance Changes in Completely Curarized Muscle*

When neuro-muscular transmission was completely blocked by curarine, a nerve volley invariably gave rise to a small localized impedance loss at the

junctional region, accompanying the e.p.p. (Fig. 9). The amplitude of this change varied with the size of the e.p.p. and thus depended upon the degree of curarization. Furthermore, a second nerve volley following several milliseconds after the first produced a larger impedance change, corresponding with a larger e.p.p. (14). For an average e.p.p. of 2 mV the local impedance change amounted, on the average, to 0.06 per cent. This compares with an impedance loss of 9 per cent during the spike (of 50–60 mV). Thus the impeddance change was reduced by curarization about 5 times more than the potential change.

In order to obtain large e.p.p.'s and so improved accuracy of measurement, the muscle was soaked in Ringer's solution containing about 5 times the normal amount of calcium. With a given concentration of curarine, calcium increased the e.p.p. and at the same time raised the threshold potential required for the initiation of muscle spikes. Consequently, larger subliminal e.p.p.'s and impedance changes were recorded without any interference by spikes or contraction.

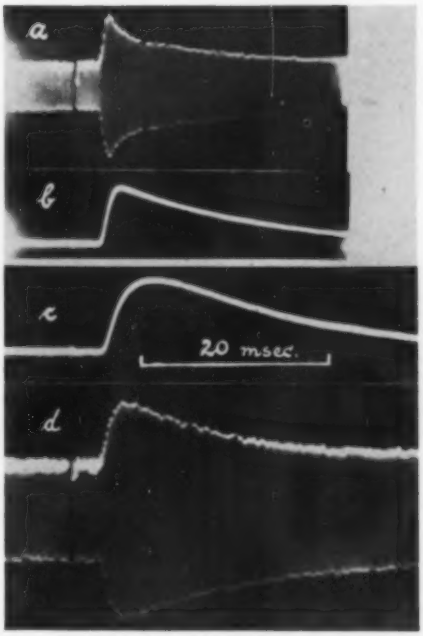

FIG. 10. Effect of eserine in currarized muscle. Calcium—Ringer. *a* and *b*: 4 μmol. curarine. e.p.p., 4 mV; impedance drop, 0.125 per cent. *c* and *d*: eserine 10^{-5} (soaked for more than 1 hour), +8 μmol. curarine (so as to reduce size of e.p.p. below spike-threshold). e.p.p., 3 mV; impedance drop, 0.08 per cent.

The time course of the impedance change showed a very consistent relation to that of the e.p.p. (Fig. 9–11). During the later parts of their decay both curves subsided at nearly the same relative rate; however the initial time courses differed, the impedance change rising and falling more rapidly than the e.p.p. In 14 experiments, at 16–28°C. (mean 22°C.), the rising period of the e.p.p. was 1.9 msec. (1.6–2.7 msec.), while that of the impedance change was only 1.05 msec. (0.85–1.45 msec.).

After application of eserine (10^{-5}), the rising periods of the e.p.p. and accompanying impedance change are lengthened two- to three-fold (Fig. 10; cf. 13).

The impedance change during a just-subthreshold e.p.p. was of the order of 0.1 per cent, though occasionally reaching 0.2 per cent (and with high calcium Ringer even 0.4 per cent). The change during a subthreshold catelectrotonic potential was considerably larger (about 0.5 per cent Section IA). This however, is only to be expected, since the myoneural junctions and, therefore, the individual e.p.p.'s, are scattered usually over a few millimetres of muscle, while the cathodal current is concentrated at the electrode, where the impedance change is measured.

It is clear from Cole and Curtis' (7) work that the actual change of membrane con-

ductance in a squid axon is much greater than the observed overall change of impedance, which is small because of the shunting effect of inert membrane components and surrounding fluid. For example, Cole and Curtis (7) estimate that, during the spike, the membrane conductance increases 40-fold (the overall impedance loss being only a few p.c.). If this estimate can be applied to muscle it would indicate that the impedance loss during a just subthreshold e.p.p., though only 1/100 of the spike change, is due to nearly 50 per cent increase of membrane conductance.

Discussion

A. *Impedance and Permeability Changes Produced by Subthreshold Currents*

As shown by the above experiments, a subthreshold current passing through an irritable membrane produces not only the well-known alterations of membrane potential (17), but in addition a local impedance change of approximately the same time course. Although no analysis at different frequencies was undertaken, it was clear that resistance and reactance changes at the cathode and during the e.p.p. had the same direction as the much larger changes accompanying the spike. Presumably they are due to the same kind of membrane alteration, which is, according to Cole and Curtis (7), an increase of its ion permeability. The question arises whether the subthreshold potential and impedance changes bear any intimate relation to the process of "excitation," that is to the discharge of the propagating potential and impedance changes.

The experiments (Fig. 5) suggest that the ion permeability of the membrane is a continuous function of the electric potential across it: the reduction of the resting potential at the cathode being accompanied by increased permeability (observed as an impedance loss), and vice versa the increase of potential at the anode by diminished permeability. According to the classical membrane hypothesis, the increased "leakiness" at the cathode would be followed by a further diminution of the resting potential; this, in turn, would cause a further increase of permeability and so on. It seems possible that in this way a progressive depolarization (and a progressive resistance breakdown) may be brought about at the cathode, such as observed during the initiation of the action potential.

After the conclusion of the present experiments, the writer became aware of the recent work by Cole and his coworkers (4, 8) in which essentially the same impedance changes were described in the isolated giant axon of the squid. The changes, at anode and cathode, were measured at various frequencies and interpreted as an alteration of membrane resistance analogous to that accompanying the spike. Moreover, the associated changes in membrane potential were recorded between inside and outside of the fibre, and their time course was found to be similar to that of the impedance changes.

B. *The Impedance Loss Associated with the e.p.p.*

The impedance loss accompanying the e.p.p. persists as long, and in its later phase declines as slowly, as the e.p.p. itself (Section II). The time scale

of this phase of decay is identical with that of the impedance and potential changes following a brief subthreshold current pulse (Section I). These observations reveal a further analogy between e.p.p. and catelectrotonic potential, and they agree with the view (14) that the descending phase of the e.p.p. is largely due to passive dissipation of a subthreshold potential change of the muscle membrane.

The difference between catelectrotonus and this part of the e.p.p. would seem to be merely that the one is produced by an artificial stimulus, the other "naturally," by a nerve volley. It is satisfactory to find that the increase of membrane permeability at the cathode can be reproduced by a motor impulse at a blocked junction, and so cannot possibly be attributed to an artifact or injurious effect of the applied stimulating current.

It remains to be asked why the initial time course of the impedance change differs consistently from that of the e.p.p. This difference, *viz.* a more rapid rise and initial fall of the impedance record, was observed in all experiments, independent of the degree of curarization (cf. Fig. 9, 10). Now it is clear from previous work (14) that, owing to the scattered distribution of individual junctions the time course of the e.p.p. recorded from whole muscle will be somewhat slower than that at the individual endplate. It might be that, for some reason, the overall record of the impedance loss is slowed relatively less than that of the e.p.p. This could be decided only if the experiment can be repeated on a single junction preparation such as described recently by Kuffler (19).

There is, however, another possibility which seems worth considering because it may provide a hint as to the mechanism by which the e.p.p. is produced. The impedance record may be due to two components: (i) one which accompanies the electrotonic change and runs the same time course as the e.p.p. (cf. above); (ii) a rapid initial component which occurs during the building up process of the e.p.p. and is superimposed on the more prolonged electrotonic change. A tentative analysis of the records on this assumption is shown in Fig. 11.

It has been pointed out (14) that the e.p.p., in completely curarized muscle, is built up by a brief transmitter action which lasts little longer than the rising phase of the e.p.p.

The relation between "transmitter action" and e.p.p. is quite similar to that between "fundamental process" and development of tension in muscle activity (15). In the present case, the time factor which causes the potential change to lag behind the "transmitter action" is due presumably to the presence of the membrane capacity.

During this initial "action period" the endplate region of the muscle is being depolarized, either by subliminal action currents from the motor nerve, or by a chemical transmitter substance (acetylcholine) which is released and removed within a few milliseconds. It is interesting to note (Fig. 11) that the "action period" described by Eccles, Katz and Kuffler (14) coincides with the rapid initial phase of impedance loss. Furthermore, both are lengthened appreciably by eserine (Fig. 10).

If the e.p.p. is due to a subthreshold discharge of the muscle membrane by nerve action currents, it would be perfectly analogous to a catelectrotonic potential set up by an applied current pulse. There is, however, no evidence for a rapid component of impedance loss concurrent with the period of extrinsic current flow; on the contrary, the indications are that the impedance change during subthreshold electric stimulation develops at the same rate

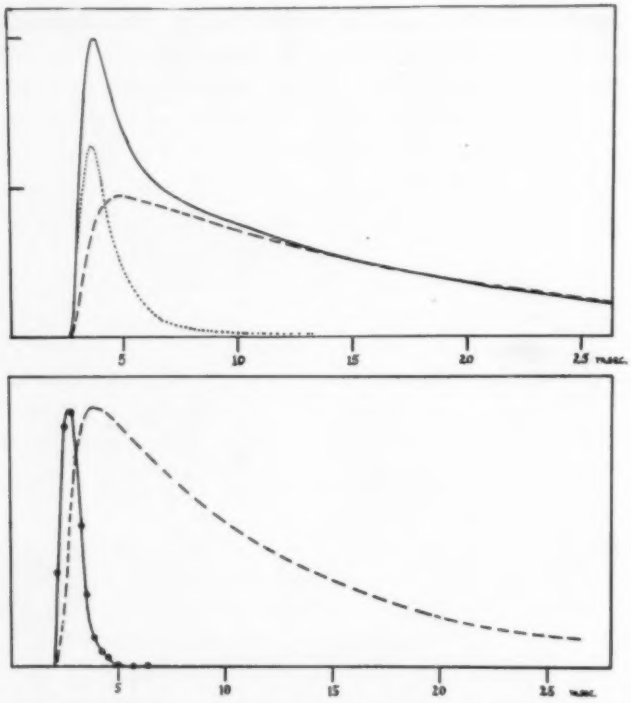

FIG. 11. *Upper part.* Full line: impedance change. Broken line: e.p.p. scaled so as to match falling part of impedance curve. The initial component of the impedance change, which diverges from the time course of the e.p.p., is shown by the dotted line. *Lower part.* Analysis by Eccles, Katz and Kuffler (14*B*, Fig. 19), showing e.p.p. (broken line) and the "probable time course of transmitter action" (full line, hollow circles).

as (cf. Section IB), or even more slowly than, the electrotonic potential (3). One might ask whether the initial impedance change during the e.p.p. is possibly due to the motor impulse and an accompanying membrane breakdown in the nerve terminals, but then one would hardly expect it to be lengthened by eserine and diminished by curarine.

On the other hand, if the depolarization of the endplate region is produced by acetylcholine, a transient diminution of membrane impedance during the "action period" might be expected. On the classical view (24), the presence of a membrane which separates different salt solutions and

restrains selectively the movement of certain ions, is responsible for the resting potential. If we accept this view, then the effect of minute quantities of acetylcholine in reducing the resting potential and making the membrane surface negative (9) would readily be explained, if acetylcholine provides a cation of specifically high penetrating power, or if it is able to render the membrane more permeable to other ions. In either event, release and "action period" of acetylcholine should be accompanied by a diminution of membrane impedance. Thus, the existence of a transient impedance loss during the phase of transmitter action, if established beyond doubt, would bear an important relation to the transmitter problem.

Summary

1. Transverse impedance measurements are made, during direct and nerve stimulation, on the isolated sartorius muscle of the frog.

2. The electric impedance of the muscle is altered at anode and cathode, following the application of a brief subthreshold current pulse. There is an impedance loss at the cathode, and a smaller impedance increase at the anode.

3. These changes decline slowly after the applied current pulse has disappeared. The time course of decay is approximately the same as that of the endplate potential and of the electrotonic potential of muscle.

4. With threshold currents, propagated spikes start at the cathode at a moment when the impedance loss reaches its peak.

5. Impedance changes set up by a nerve volley are recorded at the junctional region of the frog's sartorius. The propagated spike in normal muscle, and the endplate potential (e.p.p.) in completely curarized muscle, are both accompanied by an impedance decrease. Measuring with alternating current of 5000 c.p.s., the overall impedance loss during the spike potential (60 mV) amounts to about 10 per cent, that during the e.p.p. (2–3 mV) to only 0.1 per cent.

6. The impedance change in completely curarized muscle has the same latency and lasts as long as the e.p.p. The rising period of the impedance change is shorter (slightly more than one-half), and its initial fall more rapid than that of the e.p.p.; during the later phase of decay the time course of the impedance change is identical with that of the e.p.p.

7. The prolonged phase of the impedance loss during the e.p.p. is analogous to that produced at the cathode by a direct subthreshold current pulse. The rapid phase of impedance change, which occurs during the building up period of the e.p.p., is discussed in relation to the problem of neuromuscular transmission.

Acknowledgments: It is a pleasure to thank Dr. J. C. Eccles for his friendly encouragement throughout this work. The author is further indebted to the Carnegie Corporation of New York for a research grant and to the National Health and Medical Research Council of Australia for the maintenance of the workshop in which most of the apparatus was constructed.

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SOME PHYSIOLOGICAL ASPECTS OF AUDIOGENIC SEIZURES IN RATS*

DONALD B. LINDSLEY, FRANK W. FINGER AND CHARLES E. HENRY
Emma Pendelton Bradley Home and Brown University, Providence, Rhode Island

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SEVERAL investigators have described (11, 17, 8, 1, 6, 12, 19) an experimentally induced, abnormal pattern of behavior in the rat. The behavior is episodic and convulsive-like. It consists of sudden, violent and undirected running, jumping, stiff hopping, and tonic and clonic manifestations; this is followed by an inactive, comatose phase during which there is a lack of response to sensory stimulation, reflexes and righting responses are absent, and the animal may be molded in various positions. The active period of the attack is brief, usually lasting less than one minute; the passive phase may persist several minutes or longer, with gradual return to apparent normality.

The abnormal pattern of behavior and the conditions which evoke it were first described in detail by Maier (11), who likened it to "experimental neurosis." In Maier's original experiments the behavior was initiated by forcing a rat, by means of an air blast, to respond in the Lashley jumping apparatus to a stimulus card which it had been taught previously to avoid. The forcing of a response where available modes of response were restricted or blocked was interpreted by Maier as a "conflict" situation which led to unresolved emotional strain and "neurotic" behavior.

Morgan and Morgan (17) and others (8, 1, 6, 19) have shown that similar patterns of behavior may be evoked in the rat by various types of auditory stimulation, particularly high-pitched sounds, in the absence, presumably, of specific "conflicts." Maier and Glaser (12) produced attacks by auditory stimulation alone, but reported that "conflict" or "barriers to response" enhanced the frequency of, and susceptibility to attacks. Apparently some form of auditory stimulation, usually the hiss of air, was present in all of their experiments.

The present study[1] is concerned only with the type of abnormal behavior induced in rats by high-pitched tonal stimulation. Following the terminology of Morgan and Waldman (18) we refer to the seizure-like behavior induced by auditory stimulation as audiogenic seizures. The purpose of the study was to measure and attempt to evaluate some of physiological components of the reaction. Two physiological variables have been studied, the electro-cortical activity as recorded in the electroencephalogram and heart rate as recorded electrocardiographically.

The physiological changes and the susceptibility of the animals to seiz-

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[1] A preliminary report was made at the annual meeting of the American Psychological Association, September, 1941. (*Psychol. Bull.* 1941, *38:* 579–580).

ures were studied under the following conditions: (i) while the animal was unrestrained, *i.e.*, free to move about in a wire cage; (ii) while restrained, either physically in a special holder, or physiologically by means of partial curarization; and (iii) under conditions of delimitation of the physiological reaction system by bilateral section of the vagus nerves.

METHOD

Only rats found previously to be susceptible to audiogenic seizures were selected for study. Auditory stimulation was provided by a Galton whistle activated by an air compressor under about twenty pounds of pressure. The whistle was set to produce a tone, the major component of which was in the range of 13,000 to 14,000 cycles per sec. The whistle was within 12 to 18 in. of the animal at all times. The testing took place in an electrically shielded, sound-proof room. Two observers were present, one turned on and off the auditory stimulation at intervals, a stimulation period lasting approximately two minutes, the other observer signalled all movements and kept a descriptive protocol of the animal's activities. Electroencephalograms (EEG) and electrocardiograms (EKG) were recorded continuously in an adjacent room.

EEG and EKG were recorded by means of a four channel, Grass inkwriting oscillograph. For recording brain potentials two small, silver disc electrodes were attached by means of collodion, about 1 cm. apart, to the shaved scalp, one over the mid-line frontal, the other over the mid-line occipital region. EKG were recorded from electrodes attached to the two sides of the thoracic cavity. Despite vigorous movements during a seizure little difficulty was encountered in maintaining satisfactory electrode contacts. Light, rubber-covered wires attached to the electrodes did not interfere with the free movement of the animal about the wire cage, which was 12 in. in diameter and 14 in. high.

When animals were restrained physically they were held in a light, adjustable metal holder of cylindrical shape which fits snugly around the body wall. Front and hind legs were held in extension by means of strings attached to the paws. The head was held rigidly in a vice-like rubber lined holder and by means of thread attached to the teeth. In a few cases animals were bound, straight-jacket fashion, with gauze bandage and adhesive tape.

For producing physiological restraint a dilute solution of curare was injected in a leg vein; this caused only partial paralysis and artificial respiration was not necessary. The animals were able to move slightly with difficulty, but not vigorously, until the effects of the curare had worn off. Usually they lay on their stomachs and were unable to stand and walk. Vagotomies were performed under deep ether anesthesia by sectioning the vagus nerves in the cervical region; the animals were then allowed to recover from the anesthesia and were tested for the first time 12 to 24 hr. later.

A brief description of a severe, but essentially typical, audiogenic seizure was presented in the introduction. Although the seizures observed in different animals, and frequently in the same animal at different times, were not always of the same intensity, duration, degree of completeness of pattern, and latency of onset there was however a considerable degree of similarity. In the tabulation of the following results any animal that showed an active phase (violent running, hopping or convulsions) and a passive phase (fixed posture and quiescence or a comatose condition) while free to move about in the cage was said to have had an attack. Similarly for restrained animals any persistent struggling attempt accompanied by a comatose condition was to have been judged equivalent to an attack; as the results show no such attacks occurred.

In addition to the above seizure criteria it was observed that 95 per cent of all animals (both seizure and non-seizure) in this series gave evidence in the majority of non-seizure trials of what we have called "substitute behavior." This consisted of rapid nose or ear rubbing with one or both front paws, teeth chattering, chewing, shivering, shaking, crouching, vibrissae twitching, and restless head or other body movements. One or more of these forms of behavior appeared after the onset of the stimulus and ceased when the tone was turned off.

RESULTS

In attempting to record EEG and EKG during attacks it was thought advisable to reduce the vigorous movements of the animal by physical re-

straint; when this was done no attacks occurred in otherwise susceptible animals. This led to more extensive study of the effect of restraint, both physically and physiologically. Also because of suspected autonomic factors it was decided to test susceptibility of vagotomized animals.

Susceptibility to audiogenic seizures. Table I presents a summary of the results for all animals, showing the susceptibility to audiogenic seizures under the four test conditions. Approximately half of the unrestrained animals had seizures but only 37.5 per cent of their stimulation trials were effective; usually in the non-effective trials some form of "substitute behavior" (as described above) appeared. None of the 14 physically restrained animals had seizures, although 6 of the same animals had seizures when tested in un-

Table I. Summary of results of auditory stimulation of animals with a past history of audiogenic seizures when tested under different conditions

Test condition	No. of animals	Total no. of stim. trials	Per cent animals having seizures	Per cent stim. trials resulting in seizures	Per cent successful stim. trials in animals having seizures
Unrestrained	29	182	48.3	18.1	37.5
Restrained	14	41	0.0	0.0	—
Curarized	3	20	0.0	0.0	—
Vagotomized	2	7	0.0	0.0	—

restrained trials preceding or following the restrained trials. No overt signs of seizure activity and no characteristic EEG or heart rate changes occurred in 3 partially curarized animals which prior to, and after recovery from, the curare had seizures. Stimulation did not evoke seizures in the two vagotomized animals, although prior to vagotomy seizures were produced in both animals. One of these animals was tested on the second and third days following vagotomy with negative results. Failure to secure seizures in the vagotomized animals was also associated with a lack of heart rate change such as was typical for them during stimulation which induced seizures prior to vagotomy.

Electroencephalographic results. EEG were recorded in 13 rats during 86 stimulation trials; 8 of the rats had a total of 26 seizures. EEG changes similar to those shown in Fig. 1 and the upper half of Fig. 2, and described below, were observed in each instance that a seizure was induced. The abnormal patterns of activity in the EEG consisted of large slow waves and spike-and-slow wave complexes; these developed just before or simultaneously with the onset of the attack, usually within less than one minute after the onset of the auditory stimulus.

The character of the abnormal activity varied with the phase of the attack; large slow waves or spike-and-slow waves of 2 to 4 per sec. were associated with clonic phases; there were series of spikes or fast low amplitude waves during tonic periods; during comatose periods there was a tendency

toward absence of all electrocortical activity at first, but with a gradual return of the EEG to a normal pre-stimulus level as the animal recovered from coma. The patterns and sequence of changes in the EEG during audiogenic seizures in rats have features in common with the EEG of epileptic patients during attacks and also of non-epileptic patients during seizures

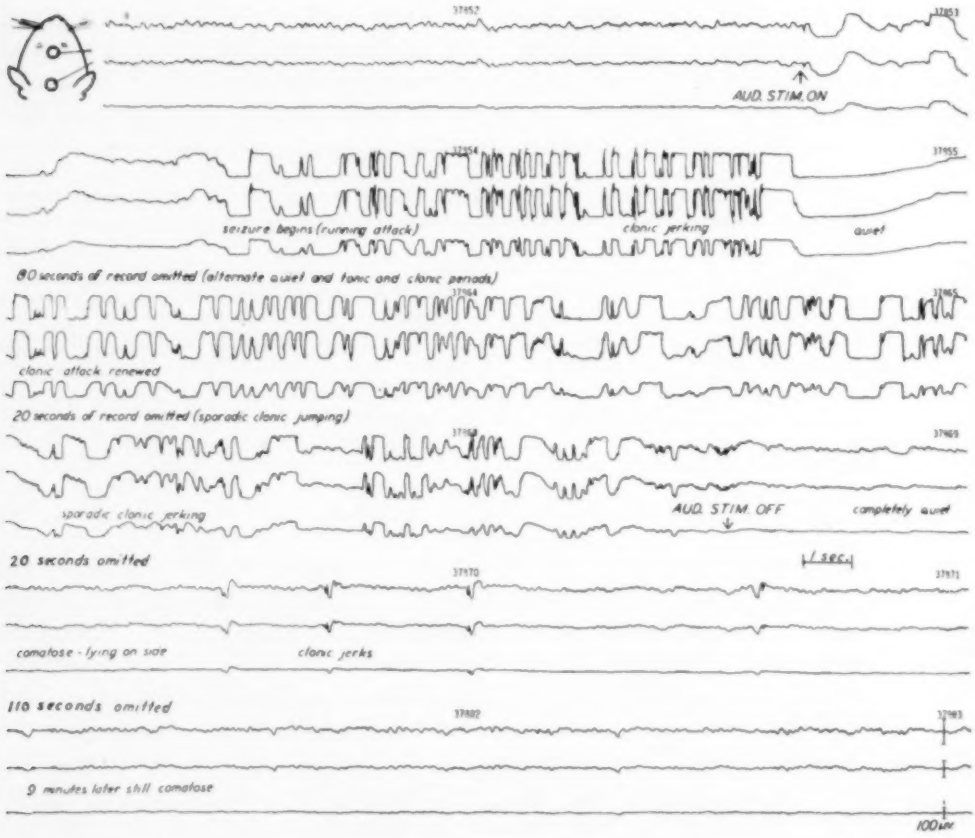

FIG. 1. Electroencephalogram (3 tracings of different amplification) of a rat during an audiogenic attack. (See p. 187).

induced by metrazol or electro-shock. This is especially true of the post-convulsive comatose phase.

Stimulation trials which did not produce seizures, either in restrained (see lower half of Fig. 2) or unrestrained rats, showed no evidence of the marked electro-cortical changes which accompanied seizures, although at the beginning of stimulation there was frequently a 5 to 10-second suppression of normal rhythms which resembles that seen in human subjects when emotionally tense or startled. There was no evidence of "sub-clinical" attacks, *i.e.*, abnormal EEG patterns of convulsive character without overt signs of seizure.

Description of Fig. 1 and 2. Figure 1 shows the EEG (three tracings with different amplification) of an unrestrained rat during a seizure. Normal rhythms prior to stimulation range from 4 to 7 per sec. and are of relatively low magnitude. With onset of stimulation the resting rhythms disappear and irregular slow waves emerge. Eight seconds after the stimulus large slow waves and spike-and-slow wave complexes ranging from 2 to 4 per sec. suddenly appear with the onset of the attack; the attack lasted almost 2 min., terminating with the cessation of the stimulus tone. Just prior to the cessation of the stimulus the

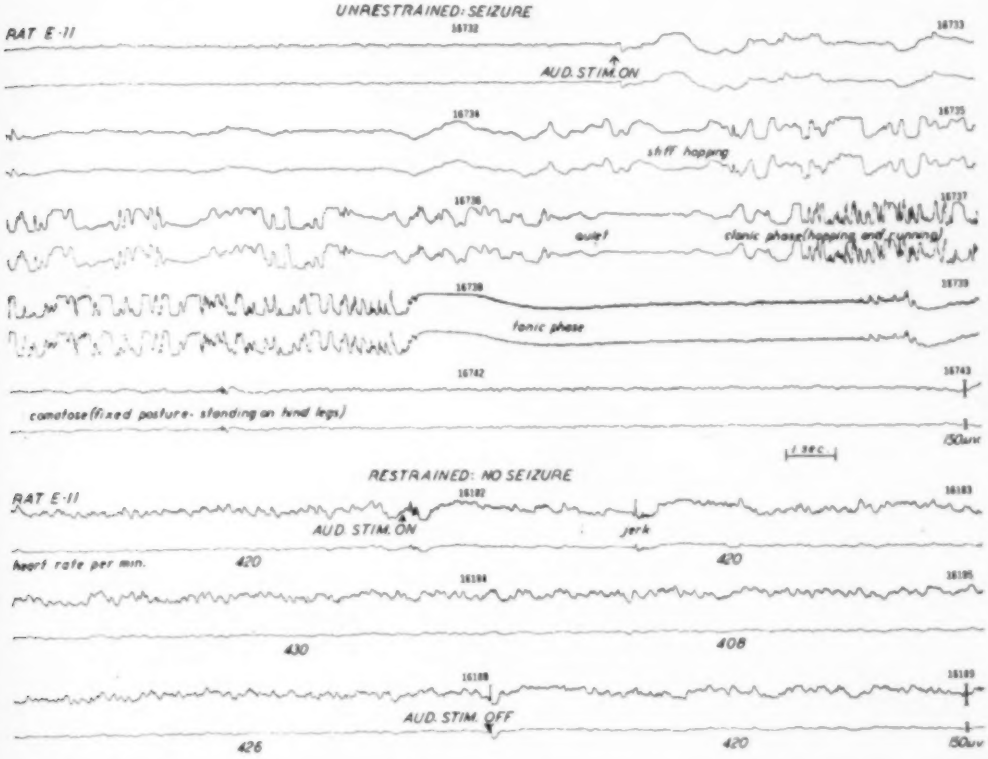

FIG. 2. Electroencephalograms (2 tracings of different amplification) of the same rat during auditory stimulation which evoked an attack (top) when animal was unrestrained, but failed to do so (bottom) when animal was restrained. (See p. 188.)

EEG disturbance abated and the animal sank to the floor of the cage in a comatose condition, remaining thus for more than ten minutes. During the comatose phase electro-cortical activities were at a minimum, pre-stimulus rhythms were barely visible at first, but gradually returned with time. Occasional isolated clonic jerks occurred during this phase; these were associated with single-spike-and-slow wave complexes.

The upper half of Fig. 2 shows the EEG (two tracings with different amplification) of another rat during an unrestrained seizure trial. Irregular slow waves appeared at the onset of stimulation. Twenty seconds later the pattern of activity in the EEG changed markedly and the attack began with stiff hopping behavior; this was followed by a brief quiescent period and then a clonic phase consisting of hopping and running; a tonic phase followed and finally a semicomatose condition during which the animal maintained a fixed posture, standing on hind legs with forelegs against the wire cage. Variations in the pattern of the EEG are associated with tonic, clonic and quiescent periods during the attack.[2] The lower

[2] The EEG are difficult to appraise since some of the electrical activity recorded is due undoubtedly to artifacts introduced by the vigorous movements of the animal. Control

portion of Fig. 2 shows an EEG from the same rat during a restrained stimulation trial which did not produce a seizure. Except for a 5-second period immediately following the onset of the stimulus, during which the normal pre-stimulus rhythms were abolished, no marked abnormalities of the EEG appeared and there was no attack. Inserted heart rate values show that there was no pronounced change in heart rate at onset or cessation of the stimulus.

Electrocardiogram and heart rate changes. Early in the study pronounced changes in heart rate were observed before, during and after the active phase

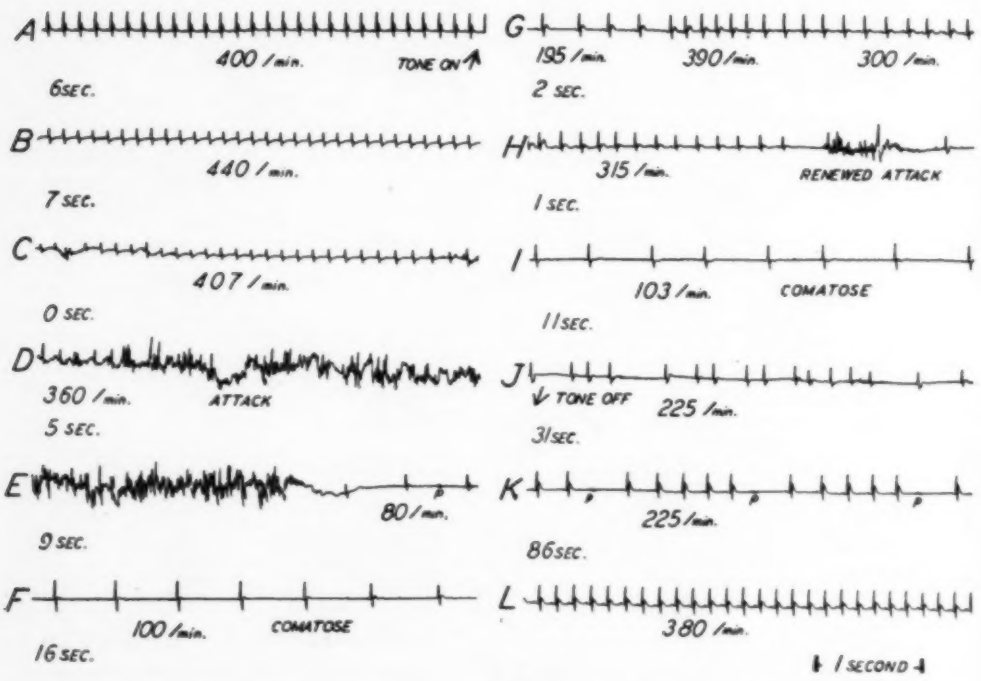

FIG. 3. EKG records before, during and after an audiogenic seizure. Note change in EKG and rise in heart rate after stimulus (B) with subsequent (C and D) decrease just prior to attack (D and E). Profound slowing of HR occurs during comatose phase following attack (F). In (G) HR increases prior to a renewed, abortive attack in (H). HR is slow, and irregular during comatose state in (I, J and K); QRS or ventricular component blocked on some beats in (K), only P-wave is present. In (L) HR returning to pre-stimulus value with recovery from comatose state approximately 3 min. after end of attack. Time intervals between sections of record indicate omitted portions.

of an attack. Whether a seizure occurred or whether only a form of "substitute behavior" (nose or ear rubbing, teeth chattering, etc.) appeared there was almost invariably, following the onset of the stimulus, a striking change in heart rate. In unrestrained animals, especially in those having seizures, three types of heart rate effects have been noted; these in order of their pre-

records with electrodes on other parts of the body have not shown the same kind of patterns, however. Attempts to record seizure patterns in restrained animals have not met with success since attacks were not evoked.

dominance are: (i) a sharp increase of considerable magnitude, with frequently a decrease just prior to an attack, (ii) a sharp decrease, usually followed by an increase prior to an attack, (iii) alternation of acceleration and deceleration, but usually with an over-all increase predominating. The type of heart rate change seemed to bear a consistent relationship to the level of heart rate at onset of stimulation: with initial heart rate at 350 to 400 per min. effect (i) occurred most frequently; when heart rate was initially high

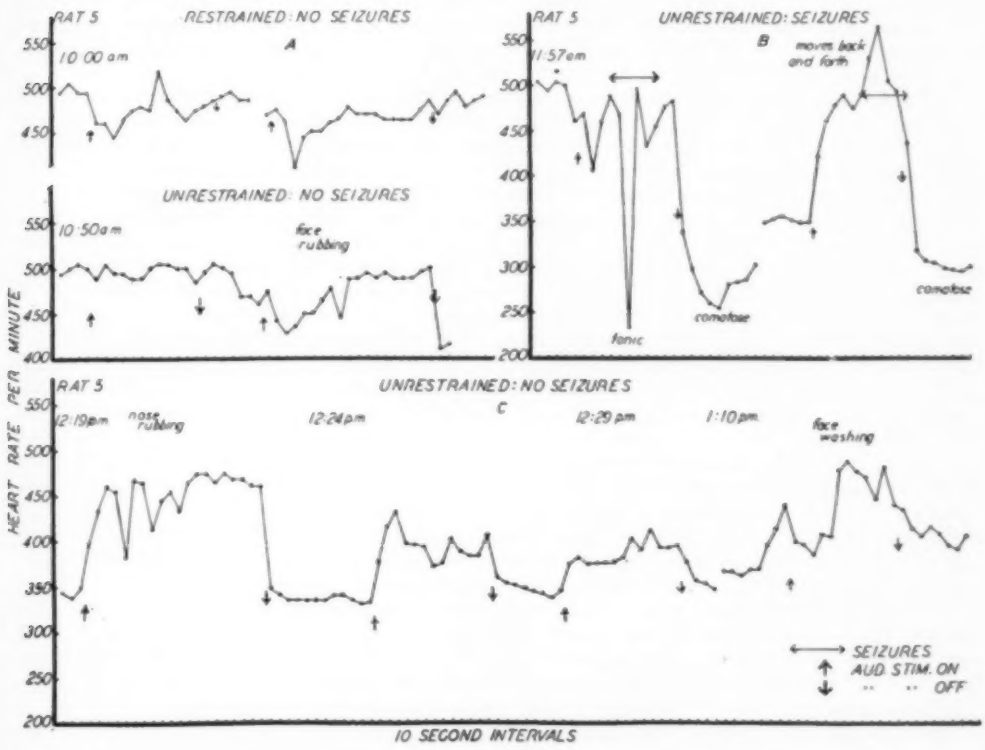

FIG. 4. Heart rate for 10-second intervals plotted for the same rat during restrained and unrestrained stimulation trials. (See p. 192.)

(450 to 500 per min.) effect (ii) occurred. Type (i) effect is illustrated in Fig. 3, and 5A. Type (ii) effect may be seen in Fig. 4B and 5C. With cessation of stimulation a drop in heart rate frequently occurred.

During the active phase of an attack heart rate was usually elevated (in part due to vigorous activity), but with occasional marked, though brief, decelerations. With the onset of the passive or comatose phase heart rate fell sharply to 100 to 300 per min., from which level it recovered gradually. In cases of more profound slowing there was frequently irregularity of the beat and in two instances during the comatose phase frequent omission of the QRS or ventricular component of the EKG (see Fig. 3K) when the

P-wave or auricular component was present; apparently transmission of the beat to the ventricle was blocked. The marked inhibition of the heart's activity following a seizure no doubt accounts for the fact that some observers (20) have reported deaths among young animals after an attack.

Description of Fig. 4. Heart rate plotted for each ten-second interval is shown for a series of restrained and unrestrained stimulation trials in the same rat. In *A* two restrained trials at 10 a.m. evoked no seizures but caused a drop in heart rate immediately after onset of the stimulus (upward arrow); with cessation of stimulating tone (downward arrow) there was no change. Two unrestrained, no-seizure trials at 10:50 a.m. had no change in heart rate on the first trial, but a decrease of about 50 beats per min. after the stimulus, followed by a gradual increase and "substitute behavior" (face rubbing) on the second trial; cessation of stimulation caused a sharp drop of about 80 beats per min.

Figure 4B shows two successive unrestrained trials at 11:57 a.m., each of which produced an attack. In the first with heart rate high initially (500 per min.) stimulation caused a sharp drop (about 70 beats per min.) which was followed by a greater rise; the attack (indicated by horizontally directed arrows) lasted one minute during which heart rate alternately rose and fell, but during a brief tonic phase of the attack dropped sharply more than 200 beats per min. for one ten-second interval. Heart rate was maintained at a high level for 20 sec. after the active phase of the attack, but fell sharply as the animal sank into coma just as the stimulating tone was turned off. After about 5 min. heart rate had recovered partially from the comatose state and was fairly well stabilized at 350 beats per min. A second stimulation applied at this time caused heart rate to rise sharply (150 beats per min. in 40 sec.); at this point behavior premonitory of an attack (slight shifting back and forth) was noted and the attack followed, lasting one minute, during which there was a still further increase in heart rate. The stimulus was turned off before the active phase of the attack abated, and heart rate immediately decreased and continued to do so as the rat sank into coma.

Figure 4C shows an immediately subsequent series of test trials in the same rat. During the first of these a sharp rise in heart rate (100 beats per min.) occurred; there was "substitute behavior" (nose rubbing) but no seizure. Heart rate showed alternate accelerations and decelerations but was maintained at an elevated level until the stimulus tone was turned off. A second trial after two minutes of rest again gave a sharp rise of heart rate, but less than on the first trial and there was no attack or "substitute behavior"; a third trial after another minute gave a smaller rise in heart rate and produced no behavioral change. These results suggest temporary adaptation to the stimulus. A final trial at 1:10 p.m. caused a drop in heart rate, followed after 40 sec. by a sharp rise and "substitute behavior" (face washing), with a decrease in heart rate on cessation of the stimulus.

Not only did vagotomy and partial curarization prevent animals from having seizures, but under each condition heart rate was unaffected by onset or cessation of stimulation (Fig. 5). Graph *A* shows the most typical heart rate change after stimulation in unrestrained seizure trials; Graph *B* the lack of heart rate change in a vagotomized animal during two stimulation trials on the 2d and 3d days following the operation. Graph *C* shows heart rate change and a seizure prior to a stimulation trial under curare in which no heart rate change or seizure occurred; graph *D* two stimulation trials under curare with no heart rate changes or seizures, whereas a third trial about 6 hr. after curare, when the paralyzing action had worn off, caused a sharp rise in heart rate and a seizure of 3 min. duration.

A quantitative analysis was made of heart rate changes for all animals in which satisfactory EKG records were obtained under restrained and unrestrained conditions; the results are shown in Table II. The average heart rate

for 3 ten-second intervals immediately before and after onset of stimulation, and immediately before and after cessation of stimulation was determined and group averages for various test conditions were computed. It will be noted that unrestrained animals show an average increase in heart rate at onset of stimulation whereas restrained animals show a decrease; at cessation of stimulation the unrestrained animals show a much greater average de-

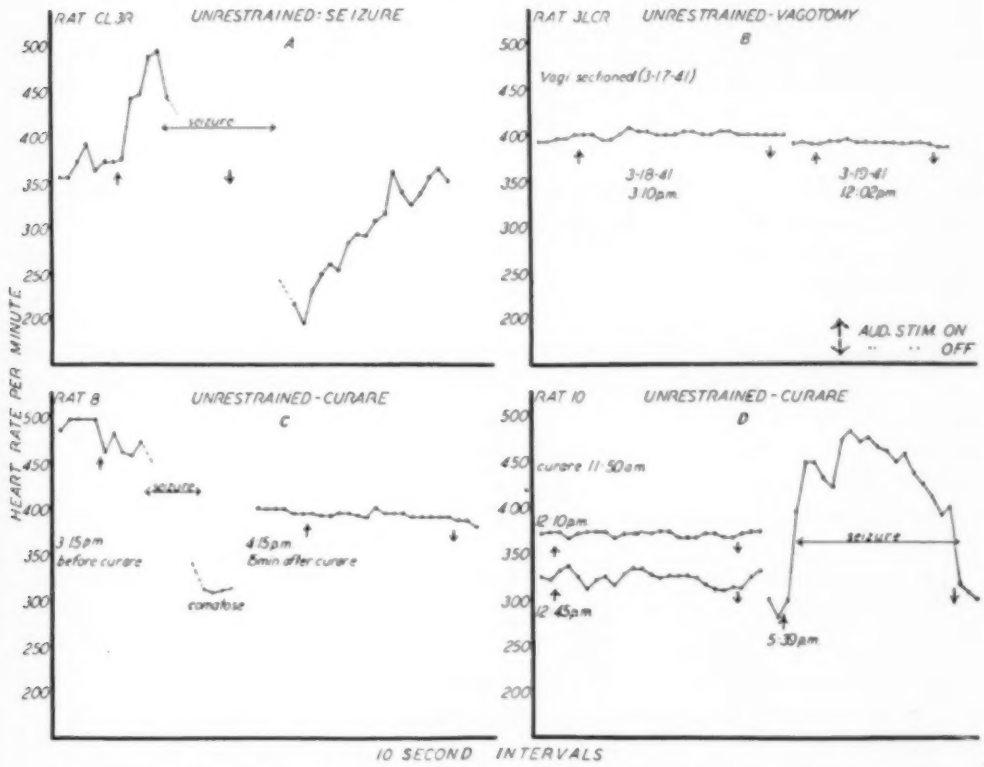

FIG. 5. Heart rate for 10-second intervals plotted. (A) Typical HR changes in unrestrained seizure trial; (B) shows lack of HR changes in vagotomized animal on stimulation; (C) and (D) show lack of HR changes in partially curarized animals during stimulation. (See p. 192.)

crease in heart rate than do the restrained animals. These differences for the same 11 animals tested under both conditions are statistically significant (by Fisher's t-test) at the 1 per cent level of confidence. When the 23 unrestrained animals are divided into seizure and non-seizure groups it is noted that a greater increase at onset of stimulation and a greater decrease at cessation of stimulation occurs in the seizure group; these differences are not statistically reliable, however.

The number of rats in each group which showed an increase, decrease, or no change in heart rate at the onset of stimulation is indicated at the right

Table II. Changes in heart rate produced by onset and by cessation of auditory stimulus and number of animals showing increase or decrease in heart rate at onset of stimulation. Heart rate based on average for three ten-second intervals before and after onset and cessation of stimulus

Test condition	No. animals	No. simulations	Heart rate per minute						No. animals showing heart rate changes at stimulus onset. (No. seizures)		
			Onset stimulation			Cessation stimulation					
			Before	After	Change	Before	After	Change	Increase	Decrease	No change
Unrestrained	23	124	390.9	401.4	10.5	396.5	373.5	−23.0	12 (7)	9 (2)	2 (0)
Restrained	12	30	460.1	455.3	−4.6	448.6	445.4	−3.2	4 (0)	7 (0)	1 (0)
Unrestrained	11	70	390.8	413.1	22.3	425.8	387.0	−38.8	8 (4)	1 (0)	2 (0)
Restrained	11	27	472.3	466.8	−5.5	456.9	453.6	−3.3	3 (0)	7 (0)	1 (0)
Unrestrained Seizure	9	44	379.4	399.5	20.1	381.3	346.3	−35.0	7 (7)	2 (2)	0 (0)
Unrestrained Non-seizure	14	70	398.2	402.6	4.4	404.8	391.0	−13.8	5 (0)	7 (0)	2 (0)

side of the table. It will be noted that a greater number of seizures (number in parentheses) occurred among rats showing an increase than in those showing a decrease in heart rate.

The differences in the heart rate averages prior to stimulation for the restrained and unrestrained animals are statistically reliable; for the matched groups of 11 animals it will be noted that initial heart rate was 81.5 beats per min. higher in the restrained animals. A difference of 18.8 beats per min. between initial heart rate averages for seizure and non-seizure unrestrained animals is not statistically significant; it is interesting however that the tendency is for the non-seizure animals to have the higher initial heart rate.

Discussion

Although auditory stimulation appears to be the chief component, rather than a neurotic reaction to "conflict," in the production of a violent, seizure-like pattern of abnormal behavior in the rat, no adequate explanation for the phenomenon has been advanced. So far only rats and mice (2) have been found susceptible to audiogenic seizures.[3] Increased intensity of the sound has been shown by Morgan and Waldman (18) to be related to increased frequency of attacks, but Morgan (15) has found that intensity and other characteristics of the stimulus are not related to the latency of attacks. According to Morgan and Gould (16) the most effective frequencies are those above the audible range for human subjects, although frequencies as low as 10,000 cycles per sec. have been found effective. Smith (20) has called attention to the cyclic character of the different phases of the attack, and has suggested that this may be due to a cortical physiological timing mechanism.

The results of the present study indicate two important underlying physiological conditions for an audiogenic attack. These in order of apparent temporal sequence are: (i) an autonomic discharge, as evidenced by heart rate changes, of both sympathetic and parasympathetic nature but with the former tending to predominate; (ii) a marked electrocortical discharge as evidenced by the convulsive-like patterns of the EEG at the onset of the attack. The autonomic discharge may occur without the electrocortical discharge and consequently without the seizure, but usually only if some form of "substitute behavior" (restless motor activity in the form of teeth chattering, nose rubbing, etc.) intervenes. Thus the auditory stimulus appears to excite autonomic centers in the diencephalon which in turn discharge both peripherally and centrally. The central discharge presumably serves to build up a cortical excitatory state which if not drained off by restless motor activity or "substitute behavior" will eventually culminate in an explosive, convulsive-like electro-cortical discharge similar to that seen in epileptic patients during a seizure or in non-epileptic patients undergoing

[3] We have found that Galton whistle or beat frequency oscillator stimulation within the upper audible ranges applied to two convulsive patients and two non-epileptics did not produce seizures or EEG signs of seizure-activity.

metrazol[4] or electro-shock treatments. The sensory influx arising from peripheral autonomic activity may serve to reinforce the central excitatory state of the cortex; a lack of seizure responses in vagotomized animals may be an indication of this. Likewise in curarized animals lack of effective autonomic (and somatic) responses may prevent the necessary sensory reinforcement sufficient to produce electro-cortical discharge.

Of particular interest is the apparent simultaneous activation of autonomic centers with the resulting competition of sympathetic and parasympatic systems, the former tending to predominate. Gellhorn and collaborators (5, 10, 3, 4) have demonstrated in rats, cats and man that emotional states are accompanied by activity of both vago-insulin and sympathico-adrenal systems, the latter predominating.

No completely satisfactory explanation has been offered for the fact that animals which are completely restrained[5] do not have seizures. First thought might suggest that they would be more susceptible to attack than unrestrained animals since opportunity for "substitute behavior" and other modes of response are limited. On the other hand these animals struggled and exercised much during the restraining process and undoubtedly continued to make essentially isometric contractions against restraint. Also their heart rate is initially higher than that of unrestrained animals. These factors might be interpreted as affording adequate outlets for nervous energy which would prevent central over-loading and consequent seizure discharge. In this connection Humphrey and Marcuse (9) have found that neuromuscular activity (exercise in an activity wheel) prior to or during stimulation reduces the frequency of attacks.

The importance of the autonomic component of the audiogenic reaction is further illustrated by preliminary results reported by Humphrey (7) and also work by Martin and Hall (14). The former found that atropine, a parasympathetic depressant, diminished the frequency of attacks in rats, whereas nicotine, a ganglionic excitant, increased the frequency of attacks. In the latter's experiments non-emotional strains of rats had more frequent attacks than emotional rats; the lability of the autonomic system and the greater activity of the emotional animals apparently served to relieve inner tension.

How high frequency auditory stimulation comes to initiate an autonomic discharge and the subsequent seizure pattern in rats is not entirely clear. A few possibilities may be mentioned. High-pitched sounds may serve as an "*emotional*" or "*fear producing*" stimulus, yet other types of "emotional" stimuli such as electric shock have not proved effective in eliciting attacks. The high frequency sounds may serve as a *pain-producing* stimulus, possibly mechanically affecting middle ear muscles (stapedius and tensor tympani);

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[4] Maier, Sacks and Glaser (13) have reported that a subconvulsive dose of metrazol has a sensitizing effect which strongly favors the development of sound-induced seizures in rats.

[5] We have observed, as have Humphrey and Marcuse (9), that rats only partially restrained, *i.e.*, with head and legs free, will have attacks.

the autonomic effects of painful stimulation are well known. Another possibility is *direct mechanical stimulation of the auricular branch of the vagus* which supplies part of the tympanic membrane and has reflex connections with the sympathetic through the superior ganglion of the vagus. Finally, *spread of excitation* from the nucleus of the eighth nerve to adjoining cranial nerves and vagus by reflex over-flow would seem to be entirely within the realm of possibility in view of the numerous interconnections among these nerves. Delimitation of the precise mechanism involved will have to await further experimentation.

Summary

Electroencephalographic and heart rate changes have been studied in rats during stimulation with high-pitched tones which produced seizures and other abnormal behavioral manifestations. Seizures occurred in approximately one half of the unrestrained rats, but no attacks were induced in restrained, curarized or vagotomized animals. Various phases of an audiogenic attack were associated with abnormalities of the EEG similar to those observed in epileptics during an attack; in non-seizure stimulation trials the EEG and heart rate changes were not noted, except in unrestrained animals where it was noted that heart rate changes were frequently accompanied by some form of "substitute behavior." Following the onset of stimulation but prior to an attack marked increases and/or decreases in heart rate occurred; these were interpreted as evidence of dual action of the autonomic system preceding audiogenic attacks, in which both sympathetic and parasympathetic systems participated, but with the former tending to predominate.

On the basis of the results an attempt has been made to outline the temporal course of physiological events leading to the development of an audiogenic seizure.

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RESPONSES DURING REFRACTORY PERIOD AT MYONEURAL JUNCTION IN ISOLATED NERVE-MUSCLE FIBRE PREPARATION

STEPHEN W. KUFFLER
Kanematsu Memorial Institute of Pathology, Sydney Hospital, Sydney

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INTRODUCTION

IN A RECENT paper (14) it has been shown that in the isolated nerve-muscle fibre preparation of frog a nerve impulse sets up a local potential at the end-plate (the e.p.p.) which in turn gives rise to the propagated muscle impulse. The object of the present experiments was to investigate the responses which a nerve impulse sets up when it acts during the refractory period of the muscle. The way in which a nerve impulse initiates a spike in partially refractory muscle and the further course of its propagation under such conditions has been studied. The single fibre preparation proved particularly suitable for such an investigation since it gave much more clear-cut and direct evidence than did the multifibre preparations of cat's soleus or frog's sartorius (5, 6).

Refractoriness in the muscle fibre was produced in two ways: (i) By an 'antidromic' muscle impulse set up by direct stimulation, as described in section A; (ii) By a muscle impulse initiated by a nerve impulse (section B and C).

The subsequent excitation was provided in both cases by a nerve impulse. The antidromic muscle impulse passes the neuromuscular junction (n.m.j.) and presumably renders that region refractory in the same way as any other region of the muscle. The excitation by the nerve impulse on the other hand not only renders the muscle refractory by virtue of the discharge of a muscle impulse, but also produces a local potential, the endplate-potential (e.p.p.), at the junctional region of the muscle (10, 8, 3). Some difference is therefore to be expected in the time course of the recovery from refractoriness set up under these two conditions (3).

METHOD

The method of recording in the interface and in paraffin has been described previously (14). In the 'antidromic' experiments (section A) the muscle fibre has been stimulated some distance away from the endplate region and a muscle impulse was set up, travelling towards the neuromuscular junction. This direct stimulation was not practicable while the fibre was in the interface, surrounded by saline which largely short-circuited the stimulating electrode. The 'antidromic' experiments therefore were always done with the whole preparation in paraffin. Moreover, the paraffin records were to be preferred because they gave a true time course of the potential changes if allowance is made for slight slowing in the amplifier due to the high input resistance. Previous difficulties (14) were largely overcome by improvements in the dissection technique, mainly by cleaning the junctional region of the nerve entry from fragments of connective tissue and fibre rests. Even so the contact between fibre and recording electrode was not as good as when recording in the interface, where the electrode could be pressed fairly firmly against the fibre. If the fibre was

carefully dissected its resistance, when recording in paraffin, was as a rule 0.3–0.5 M Ω per mm. length and its spike potential was usually between 60–100 mV.

Results

A. Recovery after an antidromic muscle impulse

The muscle at the junctional region was rendered refractory by the propagation of an impulse (*M*), set up by a stimulus applied at the electrodes S_2 (Fig. 1). At chosen intervals after the setting up of *M* the nerve was stimulated at S_1 and a nerve impulse (*N*) was sent down towards the junction. The interaction between these two impulses when arriving at the n.m.j. at less than 1 msec. apart will be described in detail in a later paper.

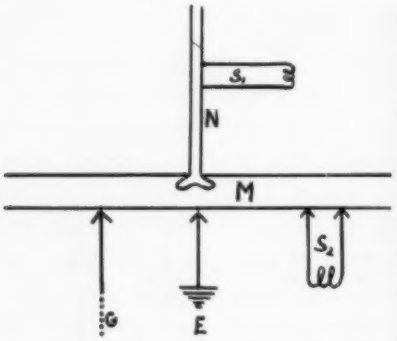

Fig. 1. Diagram of muscle fibre (*M*) with nerve(*N*). S_1, the stimulating leads on the nerve, S_2, the stimulating leads on the muscle. Recording earth lead (*E*), grid lead (*G*).

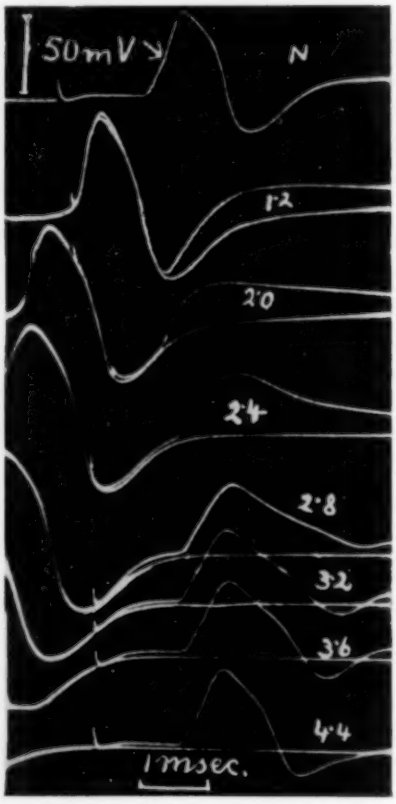

Fig. 2. Action potentials at the neuro-muscular junction set up by nerve stimulation at the indicated intervals in milliseconds after an antidromic muscle impulse (*M*). The base line in each record is formed by the conditioning *M* response alone. Top record, response to the testing nerve impulse alone (*N*) in normal muscle. Arrow indicates the initial e.p.p. step. The *N* stimulus is fired at a constant position on the sweep (see text).

Responses to the testing nerve impulse (*N*) at different stages of refractoriness are seen in Fig. 2, superimposed on the base line formed by the response to the conditioning antidromic impulse. The nerve stimulus was kept at a fixed interval after the start of the sweep, while the *MN* intervals were altered by changing the relative position of the muscle impulse (*M*).

MN interval 0.0 msec. is assumed if the muscle spikes produced by the nerve and antidromic muscle impulse would be simultaneous at the junctional region. The potentials added by *N* can be seen better in Fig. 3, where they have been plotted on the same potential-time co-ordinates, the stimulus artefact of *N* giving zero time.

The response due to nerve stimulation (*N*) in normal muscle has an initial e.p.p. step of about 50 per cent total potential height (marked by arrow in Fig. 2). Comparisons with the interfacial recordings (14) suggest that such

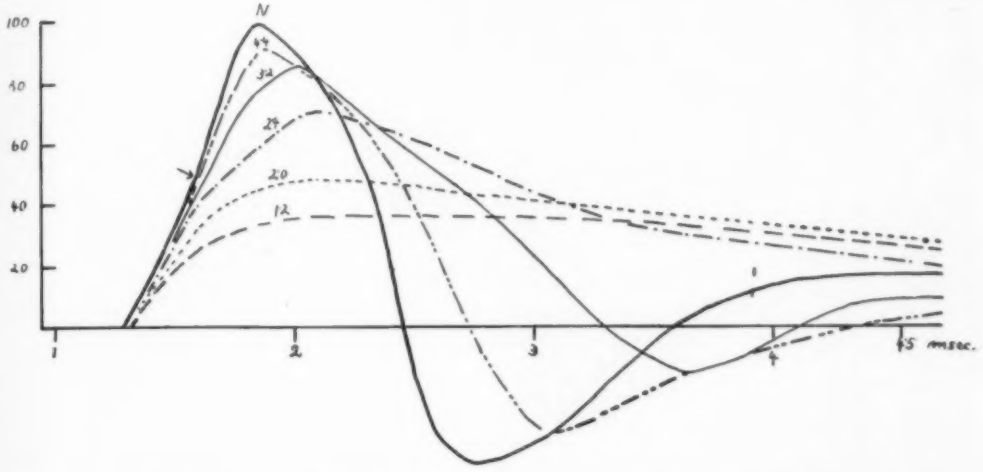

FIG. 3. Experiment of Fig. 2. Potentials, as percentage of normal peak-potential, added by *N* to *M* at the indicated intervals in milliseconds. The largest potential is a response to *N* alone, with an arrow indicating the size of the e.p.p. component. Zero time is time of nerve stimulus.

a 50 per cent e.p.p. step is due to the effective recording being about 0.2 mm. away from the endplate. The interelectrode distance between the earth lead (*E*) and grid lead (*G*) was 1.8 mm (Fig. 1).

At an *MN* interval of 1.2 msec. (Fig. 2 and 3) the absence of a diphasic wave shows that *N* has failed to set up a propagated response and at 2.0 msec. the whole potential addition is increased, but has the same time course. These are the pure endplate potentials already described in refractory muscle (6, 4, 5) and are similar to those in curarized muscle (2, 3, 14) when neuromuscular transmission had been completely blocked. With *MN* 2.4 msec. the added potential height is further increased but the time course following the peak is now appreciably different from the previous e.p.p.'s. This is due to the superposition of a small spike response on the e.p.p. An impulse had been set up, but did not reach the grid lead 1.8 mm. from the endplate. At 2.8 msec. the small diphasic wave shows that the spike response has propagated at least 1.8 mm. to the grid lead. At the longer intervals, 3.2, 3.6 and 4.4 msec., the spike and its diphasic wave increase

progressively and at the same time become earlier. The refractory period affects the response to the testing nerve impulse less and less. The earlier appearance of the diphasic wave is due mainly to quicker conduction in normal than in refractory muscle. Thus the earliest response to reach the grid lead propagates over the stretch of 1.8 mm. at an average speed of 0.9 per msec., while in normal muscle the rate increases to 2.2 per msec. Such slowed conduction had been already found in refractory nerve (9).

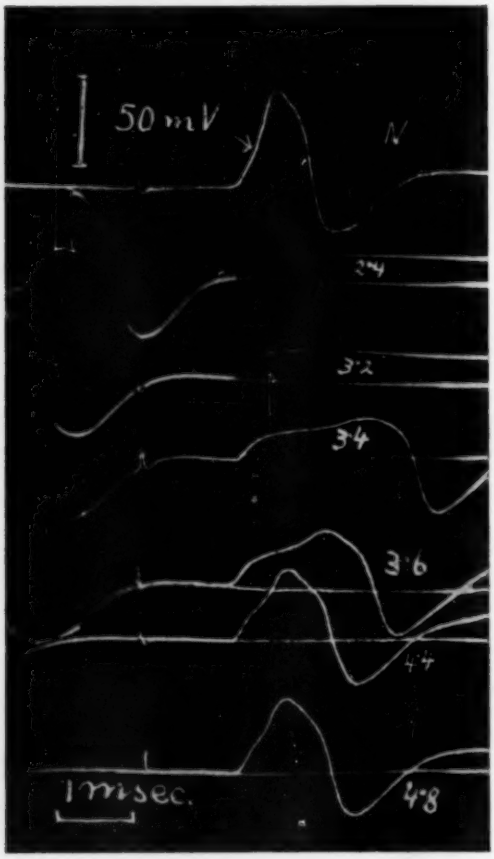

FIG. 4. Action potentials at the neuromuscular junction added by a second nerve impulse (N_2) at the indicated intervals in milliseconds after the first nerve impulse (N_1). The base line is formed by the response to N_1 alone. (At 3.4 msec. interval a base line shift has occurred.) N_2 is fired at a constant position on sweep. Arrow in top record indicates e.p.p. step in the response to nerve stimulation in normal muscle. At intervals of 3.4 to 4.8 msec. during the refractory period the e.p.p. step is more pronounced (cf. also Fig. 5.)

The smallness of the diphasic responses in partly recovered muscle, as compared with the control response (N) indicates that the impulses recorded at the grid have not yet reached their full size. In the experiment of Fig. 2 no attempt was made to investigate whether all the impulses showing a diphasic wave eventually reach their normal size and propagate along the whole muscle fibre after passing the grid lead. From comparison with other experiments it seems likely that the response at 2.8 msec. would sometimes "grow up," while at other times would die out locally (cf. Section C).

B. Recovery after nerve stimulation

A muscle impulse was initiated by nerve stimulation (N_1) and at various intervals afterwards the recovery of the muscle fibre was tested by a nerve impulse (N_2). Responses due to N_2 during the partial refractory period of the muscle are shown in Fig. 4 and are plotted in Fig. 5 to allow a better analysis. As before, the timing of the conditioning impulse (N_1) was varied while N_2 was kept at a constant position on the sweep. Zero interval for the MN series gave simultaneous M and N spike peaks (see above), hence, with similar intervals in the MN and N_1N_2 series the recovery is tested at the

same time after the conditioning spike. Moreover these two series were taken in quick succession on the same muscle fibre at a constant temperature. The earth lead was left at the endplate region, but the interelectrode distance was 1.5 mm. for the N_1N_2 series. Accordingly the first phase of the N response in Fig. 4 is the same as that in Fig. 2 while the diphasic wave appears earlier.

After a nerve impulse has been sent down to the muscle fibre, further indirect stimulation is prevented for 1.0–1.5 msec. at 18–20°C. by the refractoriness of the nerve. The earliest second nerve impulse finds the muscle

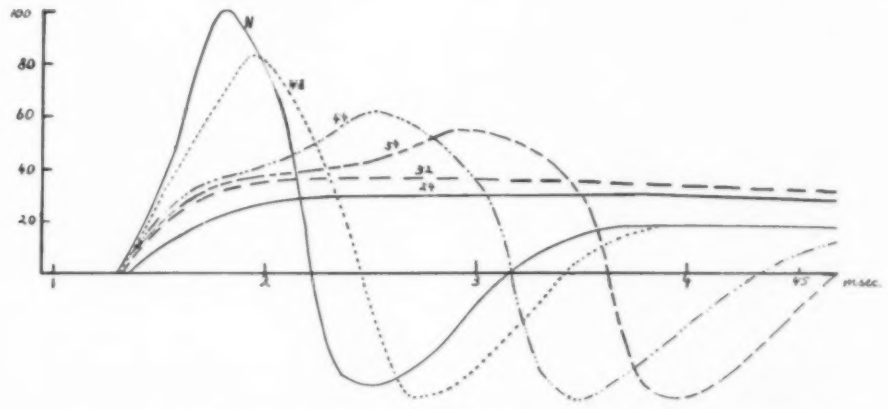

FIG. 5. Experiment of Fig. 4. Action potentials (as percentages of peak potential) added by the second nerve impulse (N_2) to first response (N_1) at intervals indicated in milliseconds (see text).

still refractory so that it cannot give a propagated impulse. Thus at 2.4 and 3.2 msec. interval only an e.p.p. is set up similar to the potentials at *MN* 1.2 and 2.0 msec. intervals of Fig. 2 and 3. A sudden change occurs at 3.4 msec., where besides the e.p.p. a small spike component appears. At the grid lead however a large diphasic wave is recorded, thus showing that at that distance from its origin the impulse has reached approximately normal size. The diphasic wave appears to be even greater than in the control *N*, but, as shown in a later paper, allowance has to be made for the background e.p.p. The further recovery from the refractory period is revealed at longer intervals. The spike component becomes bigger, appears after a shorter delay, and the diphasic wave is earlier.

In comparing the responses after antidromic (Fig. 2 and 3) with those after nerve stimulation (Fig. 4 and 5) a striking difference becomes evident. A propagated response is set up at a shorter interval with *MN* than with N_1N_2—compare the respective 3.2 msec. responses. Further, these responses at comparable intervals attain a bigger size in the *MN* series. Figures 4 and 5 also show that with the N_1N_2 series there is a much longer delay for initiation of the spike. For example at 3.4 msec. the spike peak is delayed by 1.2

msec., while with the *MN* series (Fig. 3) the delay is never longer than 0.3–0.4 msec. as compared with the control.

Thus the refractory period at the endplate region following an impulse set up by nerve stimulation is longer than the refractoriness after an 'antidromic' muscle impulse. This has already been found by Eccles and Kuffler (3) in cat's soleus and was attributed to the e.p.p. Eserine which greatly increases and prolongs the e.p.p. at the same time further lengthens the refractory period (2).

A further difference between the *MN* and N_1N_2 series is that the first propagating response after N_1 'grows up' more quickly and also propagates

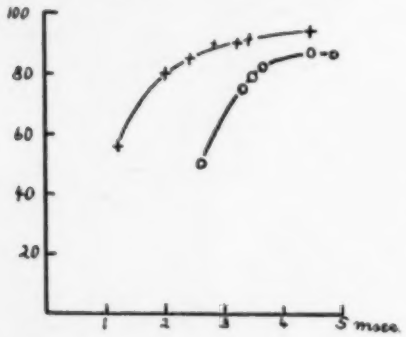

FIG. 6. Experiment shown in Fig. 2 and 4. Ordinates: the rate of rise of the e.p.p.'s, set up during the refractory period in the *MN* and N_1N_2 series, as percentage of rate of rise in normal muscle. Abscissae: response intervals in msec. Crosses, *MN* series; circles, N_1N_2 series.

at a faster rate than the first response after the antidromic. Judging from the size of the diphasic wave the 3.4 msec. response in Fig. 4 and 5 has reached its normal height 1.5 mm. from its origin and propagates at an average rate of 1.5 msec. over that stretch as compared with 0.9 per msec. at 2.8 msec. interval in Fig. 2, while the speed for normal muscle is 2.2 per msec.

In Fig. 6 the recovery of the rate of rise of the e.p.p.'s during the refractory period has been plotted for the *MN* and N_1N_2 series of Fig. 2 and 4. The larger and more prolonged refractoriness in the N_1N_2 series is well seen. At any given interval after the conditioning muscle spike the rate of rise of the e.p.p. is much steeper with the *MN* than with the N_1N_2 series.

Lengthened latent periods (shock artifact to potential rise) as seen in Fig. 5 for short intervals, *e.g.* at 2.4 msec. are usually observed and are most likely due to slowed propagation of the second nerve impulse in refractory nerve (9).

C. Abortive impulses during the refractory period

In sections A and B it has been pointed out that an impulse initiated during the refractory period at the endplate region gradually gains its full size as it propagates away from its origin. In Fig. 2 at 2.4 msec. interval an impulse was set up at the endplate region but did not propagate along the whole muscle fibre. More information about such "abortive" impulses has been obtained in the following experiments.

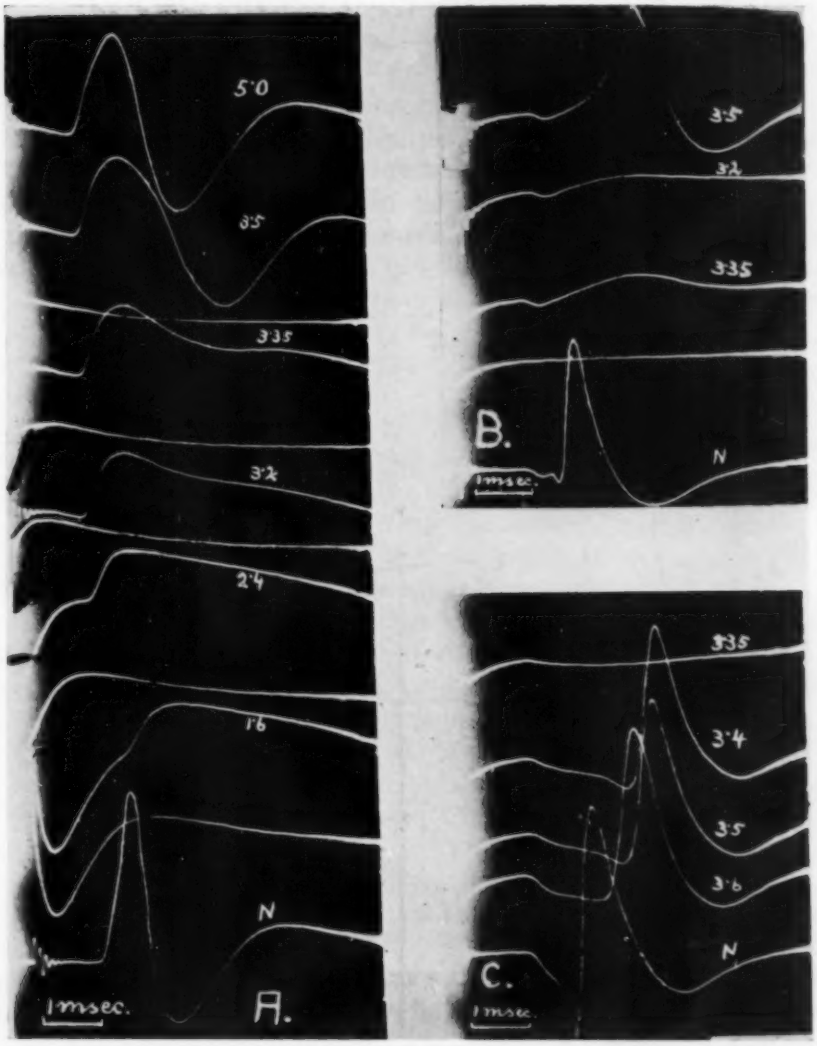

FIG. 7. Recordings in the saline-paraffin interface at different positions on the muscle fibre. Action potentials set up by a second nerve impulse during the relatively refractory period at intervals indicated in milliseconds. A base line is not illustrated in each record. The lowest records of A, B, and C, show the responses to nerve stimulation in normal muscle at different positions on the muscle fibre. A. Action potentials recorded at the endplate, B. at 0.45 mm., C. at 1.0 mm. distance from the endplate.

With interfacial recording the extent of these abortive impulses is better shown, because they produce a diphasic wave immediately they leave the region of the "endplate" electrode. Moreover, this electrode makes a more sharply localized contact with the muscle fibre and the spread of the abortive impulses can be readily determined by shifting the electrode to

various distances from the endplate. The responses stayed constant for such long periods—half an hour or more—that this method gave a reliable indication of the impulse spread.

Figure 7A shows a series of responses in partially refractory muscle with the localized lead at the endplate zone. The same series was recorded immediately afterwards 0.45 and 1.0 mm. away from the endplate and some of the critical intervals are illustrated in Fig. 7B and C. Finally, repetition of the series at the first position showed that no significant change had occurred. In the plotted records of Fig. 8, N_2 adds at 3.5 msec. interval a

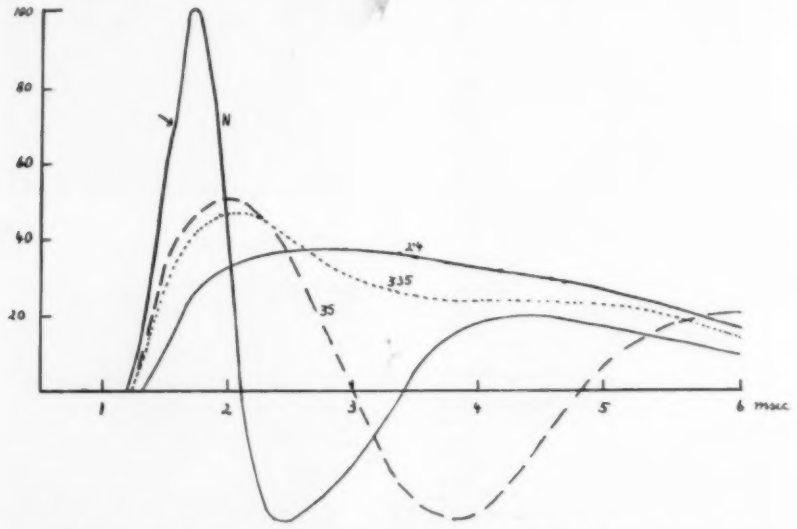

FIG. 8. Some responses of the experiment of Fig. 7A. Potentials as percentages of peak-potential. Arrow indicates e.p.p. component of rising phase.

potential with a full size diphasic response. It has set up a fully propagated impulse. At 3.35 msec. there is a small positive dip in the background e.p.p. if comparison with the 2.4 msec. response is made. The positive dip has a similar time course as the above diphasic wave. This suggests that a small impulse has propagated a short distance from the endplate and then died out. Proof for such an abortive impulse is found in the recording of Fig. 7B, 0.45 mm. away from the endplate, where a small spike is actually observed. The same interval 1.0 mm. from the endplate (Fig. 6C) does not show a spike response at all, only an inversely recorded potential.

This inverse recording is seen also in the initial positivity of propagated spikes of Fig. 6B and C, and is due to the well known effects of current distribution when leading some distance from local potentials in fibres immersed in a large conductive medium (1).

Thus it can be concluded that at 3.35 msec. interval an impulse is set up which propagates as much as 0.45 mm. from its origin and dies out be-

fore it reaches 1.0 mm. For the 3.2 msec. interval the propagation distance has not been determined accurately in this experiment; it is less than 0.45 mm., as comparison with the pure e.p.p. at 2.4 msec. (not illustrated) shows 3.4 msec. is a critical interval at which the impulse sometimes dies out and sometimes propagates (Fig. 7C). The spike peak at this and the longer intervals of Fig. 6C is appreciably later than in the normal muscle. This is due to the slowed conduction in refractory muscle (cf. Sections A and B).

In all the 28 experiments in which double *N* stimulation was used similar findings as in Fig. 7A were obtained. In 8 of these experiments it was shown, by moving the electrode, that a small positive dip following the e.p.p. peak (as in 3.2 and 3.35 msec. Fig. 7A) represents an impulse which propagates decrementally some distance, but never more than 1.0 mm.

Discussion

The present experiments have demonstrated the occurrence of "abortive" impulses during the relatively refractory period. They represent a transitional condition between blocked and normal neuromuscular transmission. Further, in partially refractory muscle the impulse gradually "grows up" as it leaves the endplate region.

The abortive impulses and the "growing up" process are not a special property of the refractory muscle. Subthreshold stimulation of normal nerve gave responses which died out locally, while with just threshold stimulation these local responses grow up to fully propagating impulses (12, 10). Moreover, similar observations have been made recently in normal isolated muscle fibres (unpublished experiments).

In the relatively refractory period the excitability of the muscle fibre is depressed and the e.p.p. has to persist longer to set up a propagated spike (Fig. 2 and 4). Further, the conditions for propagation in partially recovered muscle favor the development of "abortive" impulses and more gradual "growing up." Even under such conditions it is difficult to distinguish between the e.p.p. and the earliest abortive response, unless the latter propagates an appreciable distance. These two processes might be better separated by investigating their impedance changes, as was done by Katz (13) for the e.p.p. alone.

Although local responses can be set up by direct stimulation anywhere along the muscle or nerve fibres, they are greatly modified by the conditions under which they arise at the junctional region. Thus it appears that abortive impulses do not propagate further than 1 mm. if the refractoriness of the muscle is due to a nerve stimulus. Their size is difficult to assess, as they are recorded superimposed on the e.p.p. The total potential height may be as much as 50 per cent of the normal muscle spike potential, but about half of this is due to underlying e.p.p. When the conditioning impulse is set up antidromically responses may die out after having propagated decrementally up to 2 mm.

The eventual propagation of a small impulse is dependent on the ex-

citability gradient along the muscle fibre. Its survival depends on its ability to reach a more fully recovered and therefore more excitable part of the muscle fibre.

The action of the e.p.p. affects the excitability gradient in two ways. As shown in section B and C a cathodal lengthening of refractory period occurs (5) and thus the setting up of an impulse at the endplate region is greatly impeded. The excitability in this case gradually increases with the distance from the endplate, *i.e.* as the catelectrotonic depressant action of the e.p.p. diminishes. This explains the quick "growing up" in the double *N* series (Fig. 3). The small response reaches more and more excitable muscle as it leaves the endplate region.

On the other hand, an antidromic muscle impulse sets up a shorter refractory period on account of the absence of e.p.p. The second impulse therefore arises relatively early during the refractory period. The excitability gradient in this case will be different from above. The second impulse propagates closely behind the previous one and as it leaves the endplate region rapidly loses the support due to the catelectrotonic spread of the e.p.p. Thus in contrast with the N_1N_2 series a local response has been found to travel much further before reaching a condition at which continued propagation is assured. During the refractoriness following an antidromic impulse abortive impulses may reach up to 35 per cent of the normal spike potential height and they may propagate decrementally as far as 2 mm.

Summary

Electrical responses set up by a nerve impulse at the junctional region during the refractory period have been investigated in the single nerve-muscle fibre preparation of the M. adductor longus of frog (*Hyla aurea*).

1. Three types of responses can be recorded at the junctional region during refractoriness: (a) an endplate potential (e.p.p.); (b) "abortive" impulses (local responses); (c) fully propagated impulses.

2. During the slow propagation of an impulse away from the endplate zone in the refractory muscle the impulse may either 'grow up' to its full size and speed, or die out (abortive impulses). The dying out and the 'growing up' process occurs over 1 or 2 mm., according as the conditioning muscle impulse is set up by a nerve impulse or by direct stimulation. The abortive impulses may attain about 30 per cent of the full spike potential height.

3. The e.p.p. set up by a conditioning nerve impulse lengthens the muscle refractory period.

4. The effect of the e.p.p. in setting up muscle impulses and in modifying their spread from the myoneural region is discussed.

I wish to thank Drs. J. C. Eccles and Bernhard Katz for their valuable help and assistance during the course of these experiments and the National Health and Medical Research Council of Australia for equipping and maintaining the workshop in which most of the apparatus was made.

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EFFECT OF ESERINE ON NEUROMUSCULAR TRANSMISSION

JOHN C. ECCLES, BERNHARD KATZ* AND STEPHEN W. KUFFLER
Kanematsu Memorial Institute of Pathology
Sydney Hospital, Sydney

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INTRODUCTION

IN A SERIES of papers (19, 14, 15, 16, 17, 35, 36, 32) the properties of the "endplate potential" (e.p.p.) which a motor nerve impulse sets up at the myoneural junction have been studied in detail. The relation of the e.p.p. to the muscle membrane and its role in initiating propagated muscle impulses have been analyzed to a large extent, but the mechanism by which the e.p.p. is produced remains unknown. There are indications that the e.p.p. is set up by a chemical membrane action rather than by extrinsic currents from the motor nerve, but no decisive evidence is available. To obtain further information the effect of eserine on the e.p.p. was studied (18, 14), in particular the question how far its effects can be attributed to the protection of a transmitter substance against enzymic destruction.

The action of eserine on neuromuscular transmission has frequently been studied, but, with the exception of Feng's recent work (22), attention has been focused on the setting up of repetitive muscle responses, on Wedenski inhibition, and on localized contractions, rather than on the changes of membrane potential at the myoneural junction. There is good evidence (14, 15, 36) that the local potentials are an essential link between the transmitting agent and the setting up of muscle impulses or the production of increased or lowered excitability at the junctional region of the muscle fibre. These local potentials will, therefore, give us a more direct indication of intensity and time course of the transmitter action. The question of the repetitive muscle response and of the inhibitory effects of eserine will be dealt with in the second part of this paper, where it will be seen that they are probably entirely due to the changes in local potentials at the endplate region.

METHOD

The experiments were made during 1938–1941 on circulated cat's or isolated frog's nerve-muscle preparations (19, 33) and in a few cases on a single nerve-muscle fibre (35). The potential changes were recorded with a capacity-coupled amplifier, the fall of its response being adjusted as required by varying the size of the coupling condensers (time for one half decay being varied between 0.2 and 3.5 sec.)

Application of drugs. (a) Eserine was given intravenously to cats in doses from 25 μg. to 10 mg. per kg., combined with atropine 100 μg. per kg. The effects reached full in-

* Carnegie Research Fellow.

† Dr. Eccles' paper was posted from Australia on December 17, 1941. Owing to conditions prevailing in the South Pacific he has not been able to examine proofs. Drs. Katz and Kuffler, who have papers in this issue, were also unable to read their proofs.

tensity within 3 min. and showed a definite decline after 30 min. The frog's muscle was soaked in eserine-Ringer in concentrations of 10^{-6} to 10^{-4}. The time of soaking was usually 40 min. to 1 hr. Since eserine effects tend to diminish with rapidly repeated stimulation, standard intervals between 2 stimuli of 0.5 to 2 min. were adopted in most experiments.

(b) Curare (Merck) was injected intravenously into cats. Frog's muscles were soaked for at least 20 min. in curarine chloride (King) in concentrations of 3×10^{-7} to 5×10^{-6} in Ringer (*i.e.* according to Boehm's molecular formula about 1 to 15 μmol per l.; in our previous papers, calculations of molar concentrations of curarine were based on Boehm's formula, but it appears from recent work by King (34) on the constitution of tubocurarine that the molecular weight may be twice as high as we assumed). After 20 min. soaking a steady curarine effect was obtained even in prolonged experiments.

Results

I. The effect of eserine on the local potentials at the endplate region

A. Non-curarized muscle

1. *Effects of single nerve volleys.* The characteristic effect of eserine is an increase and lengthening of the local negative potential change which is produced at the endplate region by a nerve volley and which persists be-

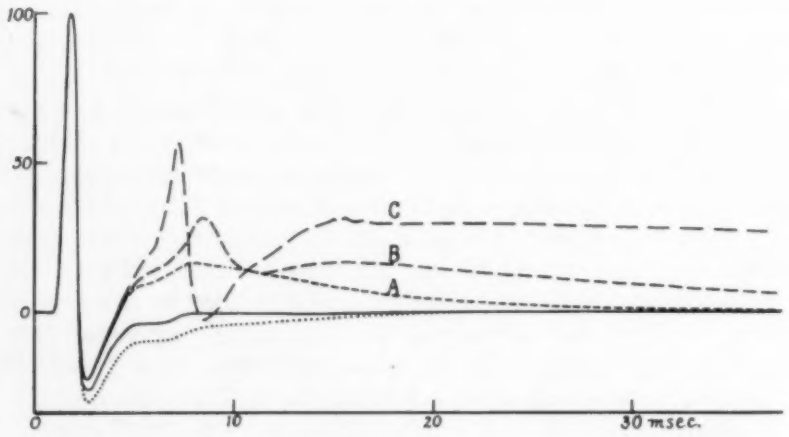

FIG. 1. Cat soleus. Action potentials set up at endplate zone by a single nerve volley. Continuous line, eserine-free muscle. Broken lines show action potentials with progressive eserinization: *A*. 150 μg.; *C*. 600 μg. eserine per kg.; *B*. 40 min. after *C*. Dotted line shows approximate time course of muscle spike potential uncomplicated by endplate potential (17). Simultaneous electrical records from nerve showed no nerve after-discharges except in *C*.

yond the initial muscle spike (17, 35, 36). The initial spike response is not changed, nor has eserine any effect on the muscle action potential set up by direct stimulation. An example is shown in Fig. 1. With progressive increase of the eserine dose, the effect becomes greater and eventually leads to the discharge of repetitive muscle spikes.

It will be shown later that the large effect in Fig. 1*C* was actually due to a cumulative action of repetitive nerve discharges, such as have been

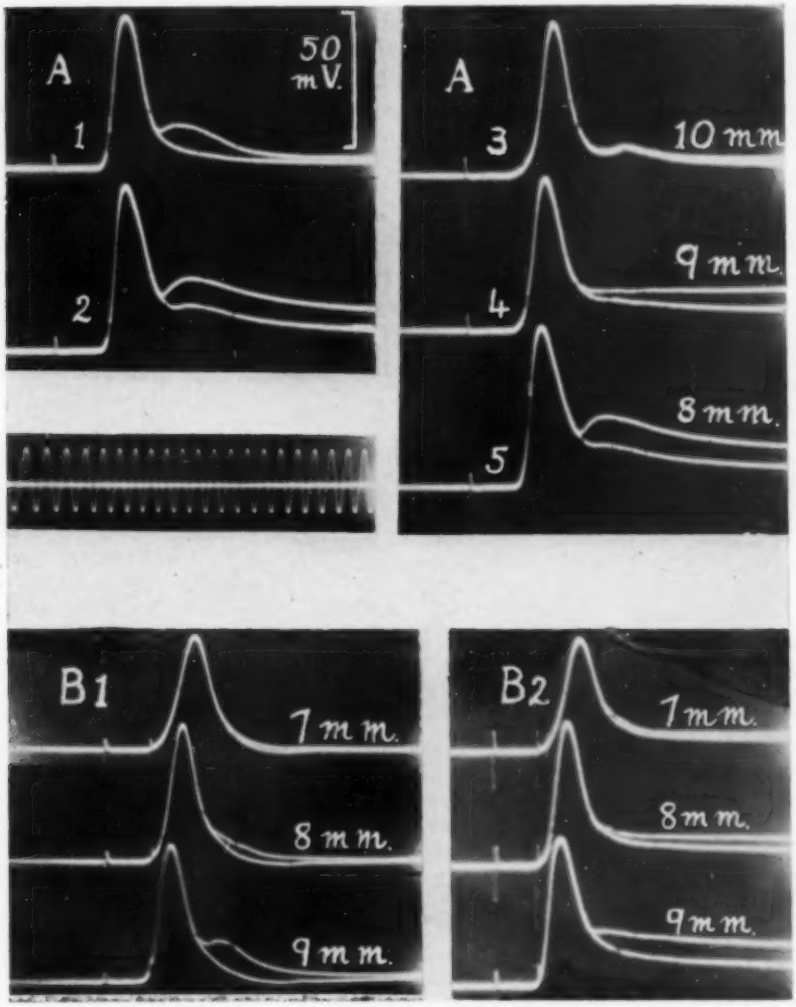

FIG. 2. Frog's sartorius, monophasic recording. Effect of eserine on e.p.p. Superimposed records of potentials at junctional region, set up by single (N_1) and double (N_1N_2) nerve volleys. *A*. 18°C. *1:* normal, N_1, with N_1N_2 at 2.8 msec. interval superimposed; N_2 adds a pure e.p.p. *2:* same, after eserine 10^{-5}. *3–5:* as 2, but N_1N_2 interval 3.2 msec., recorded at 10, 9 and 8 mm. from pelvic end respectively. Note slower rise of e.p.p. as lead is moved away from junctional region (8 mm., cf. timing of spike). *B*. 16°C. N_1N_2 at 2.4 msec. interval; position of earth lead, in mm. from tibial end, shown in figure; endplate focus at 9 mm. *1:* normal; *2:* after eserine 10^{-5}. Time signal: 1000 c.p.s.

found by Masland and Wigton (40) in the eserinized cat. However, with the smaller eserine effects, there were no nerve after-discharges, and the potentials of Fig. 1*A* and *B* were strictly due to a single motor volley.

In the frog, repetitive nerve impulses occur rarely, if ever (Section III; cf. also 23). As a consequence, the marked effect of Fig. 1*C* was not obtained

with single shocks, but could readily be reproduced by repetitive nerve stimulation (Fig. 4).

2. *Effects of double nerve volleys.* In a normal nerve-muscle preparation, a second nerve volley reaching the junction during the refractory period of the muscle sets up a pure endplate potential (e.p.p., Fig. 2; cf. 17, 19). The time course of this e.p.p. does not differ much from that of completely curarized muscle (15), *e.g.* its duration in the frog is 20–30 msec. Eserine, in a concentration of at least 10^{-5}, lengthens this potential ten times or more (Fig. 2). As will be shown later, with a sufficient dose of eserine and a brief burst of repetitive nerve volleys, the endplate negativity can be lengthened to several seconds.

Spatial spread of local potentials. Eccles, Katz and Kuffler reported (15) that the e.p.p. of curarized muscle spreads electrotonically along the muscle

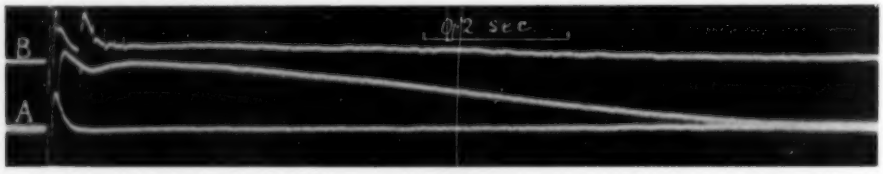

FIG. 3. Cat soleus. Action potentials at endplate zone 600 μg. eserine per kg. *A:* superimposed records set up by single nerve volley and double volley at 2.5 msec. With double volley note prolonged additional potential (the slow wave) rising from the initial e.p.p., It is slightly shortened by amplifier decay ($\frac{1}{2}$ in 2 sec.). *B.* double volley at 51 msec. sets up small prolonged potential.

membrane and that the time course of the spreading potential becomes progressively slower at increasing distance from the junction. Similarly the prolonged eserine potentials rise much more slowly at a point 1–2 mm. away from an endplate focus than at the focus itself (Fig. 2).

Facilitation of local potentials. With a moderate dose of eserine (0.5–1.0×10^{-5}) in the frog, a single nerve volley causes little prolongation of the endplate negativity, while two volleys following one another at a short interval have a large effect (cf. Fig. 2, 4*A*). This "facilitation" of the local potential is only observed if the volley interval is less than 20 msec.; with greater intervals the second volley produces *less* endplate negativity than the first. In the cat (with a moderate dose of eserine, *e.g.* 100 μg. per kg.) there is an even more striking "facilitation" of local potential (Fig. 3), but as shown in Section III below, this is due to the cumulative action of nerve after-discharges which are produced with two successive volleys at a short interval, but not with one. When there is no after-discharge, the cat differs from the frog in that the second volley always produces less endplate negativity than the first.

3. *Evidence for two separate potential waves.* In many experiments there was evidence that the endplate negativity in eserinized muscle is made up of two successive waves of depolarization, (i) a lengthened e.p.p. decaying

within 50–100 msec., and (ii) a separate "slow wave" of delayed rise and extremely slow decay (Fig. 3, 4). The slow component was brought out most markedly by (i) a relatively large dose of eserine (10^{-5}) and more in the frog, 0.5 mg. per kg. in the cat combined with (ii) a burst of repetitive nerve volleys in rapid succession.

The size of the slow wave varied in different experiments. With a dose of 0.5 to 1.0 mg. per kg. in the cat it reached as much as 40 per cent of the

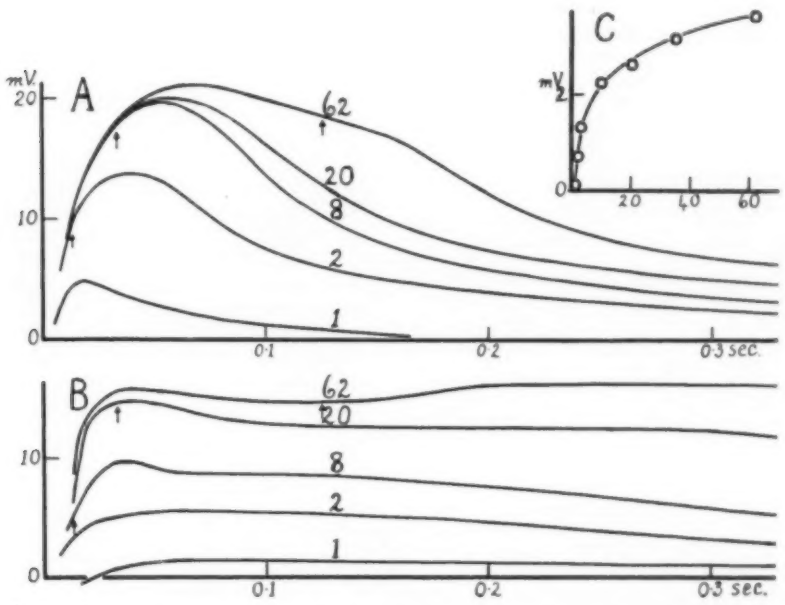

FIG. 4. Frog's sartorius. 22°C. Endplate negativity due to repetitive nerve stimulation (A.C. at 250 c.p.s.). Curves smoothed by disregarding small diphasic spikes; amplifier distortion negligible. Number of stimuli shown in fig; Arrows show end of stimulation periods with 8, 20 and 62. *A:* Eserine 10^{-5}; *B:* Eserine 3×10^{-5}; *C:* Similar experiment, 3×10^{-5} eserine. Ordinates: Height of potential, measured at 0.4 sec. after the middle of the stimulation periods. Abscissae: number of stimuli.

spike potential. At this stage a point of maximum depolarization seemed to have been reached, since it was not increased by additional nerve volleys. The separation between e.p.p. and "slow wave" was never complete, and often was only indicated by an initial phase of decline passing over to a prolonged plateau (Fig. 4*B*). Sometimes, especially with large doses of eserine, the two potentials merged completely. In the cat single or double nerve stimuli gave a large "slow wave" only when accompanied by nerve after-discharges (Section III).

In the frog, where the nerve after-discharge is absent, the slow wave could be built up by repetitive stimulation of the motor nerve. This was done by a sine-wave oscillator (250 c.p.s. giving 2 msec. intervals between alter-

nating stimuli) and the number of stimuli was varied from one or a few up to about 60.

In a normal nerve-sartorius preparation the muscle responded with large propagated spikes throughout the period of stimulation, and little or no endplate negativity was built up (see also 22). In the eserinized preparation, the propagated spike response rapidly declined during continued stimulation (22), and was replaced by a large endplate negativity with sub-

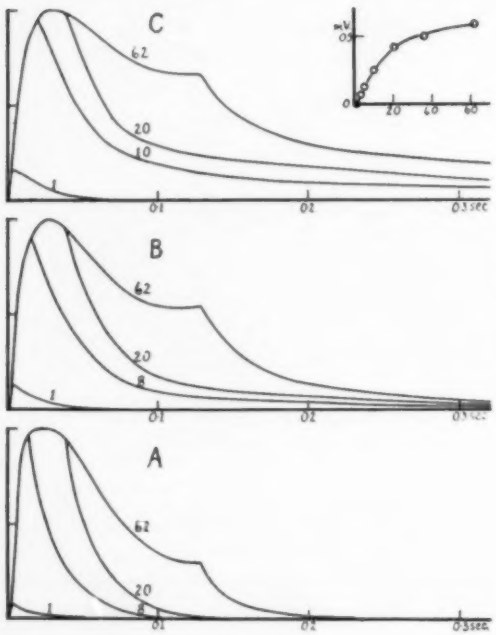

FIG. 5. Frog's sartorious. 24°C. Endplate negativity due to repetitive nerve stimulation, as in Fig. 4. Ordinates in relative units. *A:* 9 μmol. curarine, maximum potential 4.6 mV.; *B:* 9 μmol. curarine +eserine 10^{-5}, max. pot. 5.5 mV.; *C:* 9 μmol. curarine +eserine 2×10^{-5}, max. pot. 3.5 mV. *Inset:* Height of potential in *C* plotted against number of stimuli, at 0.25 sec. after middle of stimulation periods.

maximal spike discharges occasionally superimposed on it. As in the cat, there was clear evidence in several cases for the presence of two waves, an initial phase of relatively quick decline followed by a slow wave which was built up by the repetitive stimulation. A "trough" between the two waves was seen in some experiments, at 60–100 msec., the second maximum occurring at about 0.15–0.2 sec. after the beginning of stimulation (Fig. 4*B*).

It might be asked whether the "trough" was an artefact due to the muscular contraction, which might conceivably bring a region of less endplate negativity in contact with the recording lead. This was excluded because the trough was not affected by small movements of the recording electrode. Moreover, the evidence described below confirms the presence of a late potential change, even when a second rising phase could not be seen.

The relative size and time course of the two components varied somewhat in different muscles and depended upon the dose of eserine and the number of applied nerve stimuli (Fig. 4). Furthermore, with eserine doses

greater than 10^{-5}, the size of the endplate negativity became progressively smaller, while the duration of the slow wave became even greater—up to several seconds with eserine 5 to 10×10^{-5}.

In some experiments on the frog, a double potential rise was observed at rather short intervals (cf. Fig. 9 in Eccles, Katz and Kuffler, 14) with a "trough" at 5 and second maximum at 30 msec. It is not certain whether this effect was really analogous to the much slower second wave described above; it may well have been due to a combination of two potentials, one arising at endplates immediately at the recording lead, the other spreading electrotonically from an adjacent endplate focus (cf. Fig. 2).

There is evidence that with repetitive stimulation the local potentials produced by each successive nerve volley change in a systematic way. There

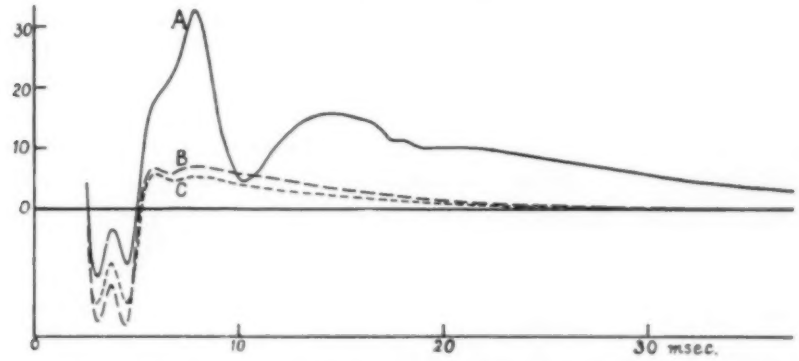

FIG. 6. Cat soleus. Action potentials at endplate zone to a single nerve volley, initial spike not shown. Ordinates: potentials as percentage of spike height. *A*, 300 μg. eserine per kg. *B* shows diminution in e.p.p. after addition of 0.1 mg. curare per kg., the potential closely resembling *C*, 50 μg. eserine per kg., no curare.

is increase with the first few volleys, then progressive decline to less than the initial size (43, 22). As a consequence the pure e.p.p. in curarized muscle was built up to a maximum with the eighth or tenth volley (in 16–20 msec.) and declined with continued stimuli (Fig. 5*A*). Similarly, the size of the slow wave in eserinized muscle, after being greatly increased by the first few volleys, received progressively smaller contributions by the later nerve volleys (Fig. 4*C*). The duration of the slow wave is so long that it has little time to decay during the relatively short period of stimulation (less than 0.12 sec.); hence, in contrast with the e.p.p., it continues to sum even with more than 50 nerve volleys, its size being roughly proportional to the integrated action of the whole series of preceding nerve volleys. During prolonged stimulation, therefore, the initial component of endplate negativity becomes progressively smaller, while the slow wave is built up to a large plateau.

B. Curare-eserine antagonism

A small, subparalytic dose of curare, if applied to a fully eserinized muscle, greatly diminishes the prolonged endplate negativity (*e.g.* Fig. 6).

With further application of curare, the eserine effect is progressively diminished until a pure e.p.p. is left, not differing strikingly from that of eserine-free muscle. Detailed investigation has shown that curare has 3 actions: (i) reducing and eventually obliterating the "slow wave" component; (ii) reducing to a lesser extent and shortening the initial e.p.p.; (iii) in the cat abolishing the after-discharge from the nerve terminals and so completely removing the striking "slow wave" effect in Fig. 3 and 1*C*.

The simplest way of studying curare-eserine antagonism is to apply graded doses of eserine to a completely curarized muscle. With a given concentration of curare (*e.g.* 6 μmol. per l. curarine in the frog), 10^{-5} eserine increased the size and duration of the e.p.p. (Fig. 7). The initial rate of rise is practically unaltered, but the e.p.p. continues to rise to a summit about 100 per cent higher and 2 to 3 times later, from which it falls somewhat more slowly than in the eserine-free muscle. This effect differs significantly from that of other agents, *e.g.* of calcium (4 to 5 times normal content), guanidine (concentration 1/50,000) or partial withdrawal of curarine (Fig. 7), all of which increase the size of the e.p.p. without appreciably altering its time course.

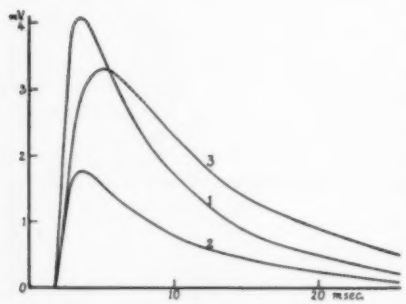

FIG. 7. Frog's sartorius. 23°C. E.p.p. incurarized muscle. *1:* after 6 μmol. curarine; *2:* after 9 μmol. curarine; *3:* after 9 μmol. curarine + eserine 10^{-5}.

The declining phase of the e.p.p. in curarized muscle has been shown to be largely a passive electrotonic decay conditioned by the electric time constant of the muscle membrane; the transmitter action is apparently much shorter than the e.p.p. which it produces (15) and decays effectively in a few milliseconds. The prolonged rising phase of the e.p.p. shows that eserine has increased the duration of the transmitter action about threefold (32). The unaltered rate of rise indicates that there is no appreciable change in the initial intensity of the transmitter action, the increase in the e.p.p. merely being due to the longer duration of this action.

When the eserine dose was increased beyond 10^{-5}, the size of the e.p.p. became smaller. At the same time its time course usually showed a slight further slowing, a maximum lengthening being reached with eserine 2 to 3×10^{-5}. Eserine had less action on the decaying phase of the pure e.p.p. than on its rising phase. Thus the average time for half decay was lengthened by about 50 per cent, *e.g.* from 8 to 12 msec. at 20°C., frog; from 3.5 to 5 msec., cat. There are three possible causes of this lengthening: (i) an effect of eserine on the electric properties and, therefore, on the time constant of the muscle membrane; (ii) a slowing resulting from the greater duration of the rising phase and a consequently greater electrotonic spread of the e.p.p. (cf. 31, p. 300); (iii) a small remainder of transmitter activity persisting during the decay of the e.p.p. and actively maintaining some depolarization.

Factors (i) and (ii) could be eliminated by the following experiments: (i) A simple index of the electric time constant of the muscle membrane was obtained by passing a small, subthreshold current through the muscle *via* 2 non-polarizable electrodes, and recording the time course of the polarization potential (32). Its average half-time is of the same order as that of the e.p.p. in eserine-free muscle (cf. also 44). Eserine had no effect on the time course of this polarization potential, showing that the electric time constant of the membrane is unaltered. (ii) According to the theory of electrotonic potential spread, some slowing of decay would occur as a consequence of the prolonged rising phase of the e.p.p. It is difficult to predict whether this effect would be large enough to account for the observed change. However, one can effectively lengthen the rise of the e.p.p. in curarized eserine-free muscle by applying several nerve volleys in rapid succession. More than a threefold lengthening of the rising phase (4 to 8 shocks at 2 msec. intervals) produced only a 10 per cent slowing of decay.

Thus, it seems most likely that in eserinized muscle the slowed decay of the e.p.p. is largely due to a small remainder of transmitting agent lingering on for many milliseconds and actively supporting the depolarization.

Neuromuscular facilitation after eserine. If the second e.p.p. exceeds a critical threshold level, the arrival of two nerve volleys in curarized muscle gives rise to propagated muscle spikes (15). In frog's muscle, this facilitation process depends upon two factors: (i) summation of successive e.p.p's, (ii) a relatively long "supernormal" period during which the second e.p.p. is greater than the first. Eserine, while increasing the duration of the e.p.p., reduces this "supernormal" effect. To quote the most striking case; with eserine 10^{-5} the maximum second e.p.p. was only 25 per cent greater than the first (as compared with an excess of 92 per cent before eserine); with eserine 2×10^{-5}, the second e.p.p. was only 6 per cent greater than the first. This diminished facilitation offsets the lengthening, and as a net result the duration of neuro-muscular facilitation remains practically unaltered by eserine, as was found by Bremer and Kleintjens (4). With the cat eserine produces no change in the relative size of the second e.p.p. (about 80 per cent, Eccles, Katz and Kuffler, 15), so facilitation is observed with slightly longer volley intervals, *e.g.* 8 msec. increased to 10 msec. Even this small lengthening of facilitation was not found by Maaske, Boyd and Brosnan (37) in dogs paralyzed by curare or magnesium.

It may be of interest to note that in the frog, after eserine, longer intervals between two successive nerve volleys were required to obtain a maximum second e.p.p. Thus, in the above-mentioned experiment, the maximum addition was obtained: (i) with curarine only, after 3 msec.; (ii) with eserine 10^{-5}, after 8 msec.; (iii) with eserine 2×10^{-5}, after nearly 20 msec.

The time course of the second *e.p.p.* was in many cases identical with that of the first; in some frog experiments, however, the second e.p.p. was more markedly lengthened than the first.

In a few experiments on the temperature coefficient of the e.p.p. (15) it was noticed that the effect of eserine (1 to 2×10^{-5}) in lengthening the e.p.p. was abolished by lowering the temperature. This observation will require further study, but it is clearly related to a previous statement by Feng and Shen (26), *viz.* that the localized contraction in eserinized muscle disappears at low temperature.

II. EXCITATORY AND INHIBITORY ACTIONS OF PROLONGED ENDPLATE NEGATIVITY

Previous work on nerve and muscle has shown a close relation between changes of membrane potential and of excitability. It has been pointed out

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(14, 15) that the effects of local depolarizations of the muscle at and around the endplate are analogous to those of cathodal currents applied artificially to nerve or muscle fibres. In a normal resting fibre the local depolarization has an excitatory effect: if exceeding a critical level, it can sum with subthreshold electric stimuli (3) and thus, for example, it speeds up the propagation of a muscle impulse (15). In refractory muscle an added e.p.p. was shown to delay the recovery process (16, 17) an effect which is probably responsible for Wedensky inhibition and analogous to cathodal effects in refractory nerve (3, 8). Furthermore it is well known that an intense and prolonged catelectrotonus blocks the propagation of impulses. The increased and prolonged local potentials after eserine would, therefore, be expected to have two opposing effects: (i) production of repetitive muscle spikes; (ii) prolongation of the local refractory period and possibly even block both of neuro-muscular transmission and of the propagation of muscle impulses. These effects have been described by several authors (5, 6, 11, 14, 17, 22, 24, 25, 42) and need not again be dealt with at length.

If a burst of repetitive volleys at 2 msec. intervals is sent into a curarized muscle (cf. Section I*A* above) the e.p.p. builds up to a peak after about 10 volleys and then declines to a much lower level, falling to about 30 per cent of the maximum in 0.12 sec. (Fig. 5*A*). At the end of prolonged stimulation the e.p.p. decays a little more slowly than after a single volley (*e.g.* 35 per cent slowing with 0.1 sec. stimulation). There was no definite evidence for a protracted "slow wave" though a trace of it, not exceeding 1–2 per cent of the e.p.p., was noticed following 0.12 sec. stimulation.

With progressive doses of eserine, a conspicuous "slow wave" component was brought out by repetitive stimulation. Its time course of decay, and rate of summation were much the same as in non-curarized muscle (cf. Fig. 4 and 5), but its size was reduced by curare to a greater extent than that of the initial e.p.p. A number of further experiments were made in an attempt to clear up the nature of the curare-eserine antagonism: their description will be included in the general discussion below.

It appears that any procedure which has deteriorating effects on the muscle membrane (*e.g.*, application of KCl, insufficient rests between stimulations) favours the appearance of "inhibitory" actions (that is effect (ii) above) and reduces or abolishes repetitive response. On the other hand, it was shown by Cowan (11) that those procedures which slow the rate of "accommodation" of frog's muscle (Ca-withdrawal, prolonged cooling of the animals) favour the appearance of prolonged repetitive discharges. This also agrees with the evidence derived from electric stimulation of isolated nerve (30).

(a) *Repetitive muscle responses.* Repetitive muscle spikes arising from the prolonged endplate negativity were frequently observed, both in the cat and frog. It is true that, in the cat, many of these repetitive spikes were associated with repetitive discharges in the motor nerve (Section III). However, simultaneous recording from nerve and muscle (Fig. 8*A*) showed that, even in the absence of nerve after-discharge, the e.p.p. following the spike gave rise to muscle impulses if it was more than about 15 per cent of the normal spike potential (Fig. 1*B*). Moreover, when the slow wave was

separated by a distinct "trough" from the initial e.p.p., the second rise of the potential was associated with a new burst of independent muscle impulses (Fig. 9*B*), which followed a silent period of about 30 msec. during the trough, and were usually unaccompanied by nerve discharge. The initial repetitive response varied considerably in different muscles: *e.g.* tibialis anticus and peronaeus tertius (Fig. 8*B* and *C*) gave a longer train of repetitive responses than soleus (Fig. 1 and 9*A*).

In the frog repetitive discharges quite similar to those in cat muscle were frequently observed, although there was no after-discharge in the motor nerve (Section III; 23). With successive stimulations the repetitive bursts declined in size and duration, while the local potentials were not appreciably diminished. In several cases, a slight injury such as produced by making the muscle "monophasic" (potassium application to one end) practically abolished the repetitive response (Fig. 2). Analogous effects have been observed with electric stimulation of nerve or muscle (30, 41), where repetitive response to constant current is greatly affected by any slight damage. It is clear that the appearance of repetitive spikes in eserinized muscle is no reliable index of the persistence of transmitter activity. They depend upon additional factors involving the excitability of the muscle fibres, their rates both of recovery and of adaptation to prolonged depolarization.

(b) *"Inhibitory" actions at the neuromuscular junction.* (i) Lengthening of refractory period by eserine. It has been shown by Eccles and Kuffler (17) that the minimum interval between two successive muscle spikes in the normal cat's soleus is about 2.2 msec. after an initial direct stimulus, and 3.0 msec. after an initial nerve stimulus. The difference was attributed to a lengthening of refractory period of the muscle by the e.p.p. outlasting the first spike (36). This effect is enhanced by the addition of a second e.p.p. early in the refractory period, and also by the application of eserine, which increases the size and duration of the e.p.p. persisting beyond the spike (Fig. 1). Thus after an initial nerve volley, the minimum spike interval in the cat's soleus muscle was lengthened by eserine from about 3.0 to 5.0 msec. The effects were similar in the frog's sartorius muscle.

(ii) The large slow wave in the cat (30 to 40 per cent of the spike potential) is accompanied by complete neuromuscular block. A testing nerve volley sets up neither muscle impulses nor local negative potential. Furthermore, Feng (21) has shown that a prolonged junctional depolarization by continued high-frequency stimulation blocks the propagation of directly excited muscle impulses past the junctional region. Presumably these effects are due to the depressing action of the prolonged intense catelectrotonus at the endplate region, and are analogous to the cathodal block in nerve first described by Werigo (46).

III. REPETITIVE DISCHARGE FROM ESERINIZED NERVE TERMINALS

Masland and Wigton (40) observed repetitive after-discharges in the motor nerve, originating from the nerve terminals of eserinized cat's

muscle. It was of interest to study the relation of these nerve discharges to the local and propagated potential changes in the muscle. This was done by recording simultaneously (i) the potential changes at the endplate region of the innervated strip preparation of soleus or peronaeus tertius; and (ii) the motor impulses in the nerve supplying this muscle, the afferent fibres having been degenerated by aseptic removal of the dorsal root ganglion of the corresponding spinal nerves at least four days previously. The motor nerve was cut at the beginning of the experiment; the recording electrodes were applied more peripherally. Repeated tests during the experiments showed that relatively weak shocks applied through the recording electrodes gave a full muscle response, hence conduction from the nerve terminals to the recording lead was unimpaired in all motor fibres concerned.

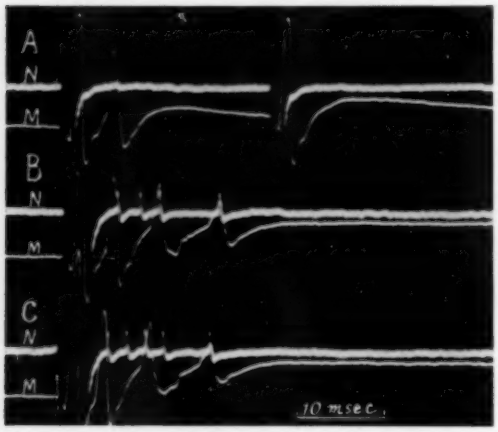

FIG. 8. Cat, peronaeus tertius, 300 μg. eserine per kg. Simultaneously recorded action potentials of endplate zone of muscle (M) and deafferented motor nerve(N) to two nerve volleys. Initial nerve spikes not shown, as amplification too high. A: no nerve after-discharges with volley interval of 24 msec.; B and C: with volley intervals of 1.2 and 1.6 msec. after-discharges of four and five nerve impulses apparently in same nerve fibre. Note more intense repetitive muscle response and more prolonged negative potential.

The finding of Masland and Wigton (40) was readily confirmed. With increasing eserine dosage, there was in many experiments a striking parallelism between the onsets of repetitive discharges in muscle and nerve fibres. In the absence of simultaneous recording the conconclusion might well have been drawn that a separate nerve impulse was responsible for every discharge of the muscle. In every experiment, however, some repetitive muscle spikes were observed in the absence of nerve after-discharges (Fig. 1B and 8A), *i.e.* the prolonged endplate negativity alone is capable of setting up multiple muscle spikes (cf. preceding section). This is quite clear also with eserinized frog muscle, where simultaneous recording from the ventral roots revealed complete absence of nerve after-discharges (confirming Feng, 23).

The cause of the nerve after-discharge remains an open question. With increased eserine dosage, impulses are discharged spontaneously from the nerve terminals (40) and cause the well-known eserine twitching. This suggests that, prior to the onset of spontaneous discharges, the nerve terminals must be in such a hyper-excitable condition that a small additional stimulus would fire them off. This additional stimulus may be provided in various ways: for example, as Masland and Wigton have shown, by an intra-arterial injection of acetylcholine, or as shown below, by the action currents of the muscle.

The retrograde effect of the muscle spike on the nerve terminals is tested by stimulating the muscle directly at some distance from the endplate zone, and recording simultaneously from junction and motor nerve. In normal, or even in unconditioned eserinized preparations antidromic muscle spikes are never found to excite motor nerve terminals. On the other hand, when the eserinized muscle (100 μg. per kg. or more) is conditioned by one or more preceding nerve volleys, an antidromic spike always evokes the discharge of nerve impulses (Fig. 9*A*), thus giving a "retrograde trans-

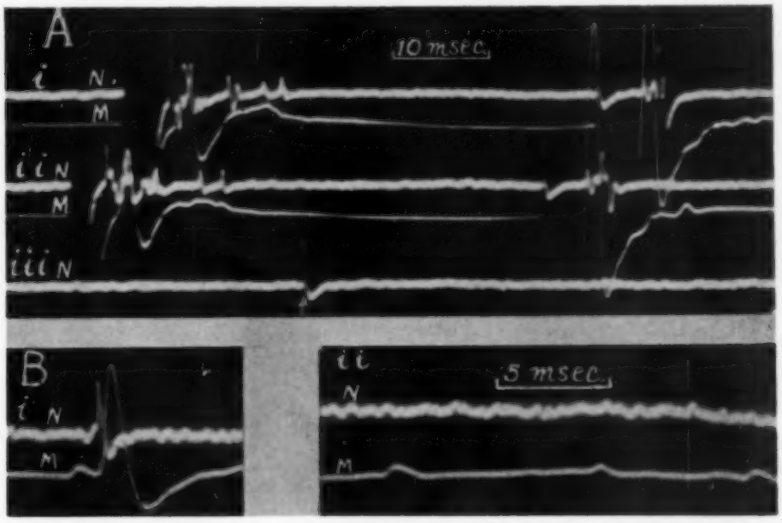

FIG. 9. *A:* Cat, soleus, 200 μg. eserine per kg. Simultaneous action potentials as with Fig. 8. Muscle amplifier has very short time constant, so so "slow wave" not recorded. *i* and *ii:* Initial nerve volley sets up repetitive muscle response and nerve after-discharge (initial muscle and nerve spikes not shown); antidromic muscle volley 49 msec. later (spike partly shown) also sets up brief burst of nerve impulses, beginning about 1 msec. after arrival of muscle spike at endplate zone. *iii:* Nerve record only, showing no nerve discharge due to antidromic volley alone (stimulus artefact shown by arrow).

B: i: "triple response" arising 50 msec. after a double nerve volley —small muscle spike followed by nerve spike, then large muscle spike. *ii:* as in *i*, but showing three small muscle spikes without the later nerve and large muscle spikes.

mission" from muscle to nerve. The possibility of nerve excitation by stimulus leak is easily excluded, for the nerve impulses are observed only after the muscle impulses have travelled from their point of origin to the endplate zone.

There is close correlation between the period of "retrograde transmission" and the duration of the negative potential at the endplate region. When a large slow wave is present "retrograde transmission" can be observed for several seconds after the conditioning nerve volley. In the ab-

sence of a large slow wave, reversed transmission is often observed for several (8–14) msec. only, *i.e.* during the decay of the e.p.p.

This "retrograde transmission" suggests that repetitive muscle spikes arising directly from the prolonged endplate negativity (Section II) would be responsible for some of the nerve discharge. Further evidence for this is given by records showing a triple sequence of action potentials (Fig. 9*B*): a small muscle action potential—possibly due to an impulse arising at a single endplate—an immediately following nerve impulse, and then the large muscle spike of that motor unit. There is no doubt, however, that some nerve discharges are independent of, and prior to, the muscle impulses (cf. the initial nerve after discharge of Fig. 9*A*). Whether these nerve spikes are set up by an electric effect, *e.g.* by the concomitant e.p.p., or by acetylcholine action (40) cannot be decided at present.

With moderate doses of aserine (50–100 μg per kg.) two nerve volleys at intervals of less than 10 msec. set up a nerve after-discharge, while there is no such discharge with a single volley. This explains the striking potentiation of endplate negativity and muscle discharges observed in the cat with two nerve volleys at a short interval (Fig. 3, 8). Even if originating at a single nerve terminal only, the repetitive nerve impulses would spread by "axon reflexes" to all the fibres of that motor unit, *i.e.* in addition to the two initial nerve volleys the whole motor unit is subjected to a brief high-frequency burst of nerve impulses. Presumably such "axon reflexes" are responsible for the sequence of small initial and large later muscle spike in the "triple responses" described above (Fig. 9*B*).

Discussion

The observations in this paper are consistent with the view that the junctional region of the muscle responds to the local depolarizations in the same way as any other region of the muscle responds to a catelectrotonic potential. If there is any special process at the junctional region, it would concern the setting up of the catelectrotonus by the transmitting agent, and it is this process which is lengthened by eserine, and diminished and shortened by curarine.

The fact that eserine lengthens the depolarizing action of the transmitter (see Section I*B* above) focusses attention on the acetylcholine (ACh) theory. It seems likely that this effect is related to the inhibition by eserine of ACh hydrolysis, and hence that ACh is the agent responsible for the junctional potential change. Some difficulties, however, arise when considering the curare-eserine antagonism (Section I*B*).

Further experiments on curare-eserine antagonism. Curare is known to oppose the depolarization of the muscle membrane by ACh (10); hence the diminution of the e.p.p. by curare provides no obstacle to the ACh hypothesis. On the other hand it is difficult to explain the drastic shortening by curare of the prolonged eserine-potential (shortening of e.p.p. and great

diminution of slow wave). The following possibilities have been tested experimentally.

1. In order to detect a possible antagonism between curarine and eserine with regard to esterase inhibition, the hydrolysis of ACh by muscle and serum esterase was measured by the manometric method (1, 45).

The manometers were filled with N_2 containing 5.1 per cent CO_2. Each vessel contained a known volume of bicarbonate Ringer (using the mixtures described by Stedman and Stedman, 45, and Marnay and Nachmansohn, 39, for dilution of human serum and frog's tissue suspensions respectively) to which was added a known volume of an enzyme preparation—human blood serum, or a fine suspension of frog's nerves or innervated portions of sartorius and semitendinosus muscles. In the example of Fig. 10, muscle suspensions were used, each flask containing 170 mg. in 3 ml. solution. The contents of the 3 flasks were made up as follows: *A*, Muscle in 3 ml. bicarbonate Ringer; *B* as *A*, but containing eserine 10^{-5}; *C* as *B*, but containing in addition 7.5 μmol./l. curarine. The side bulb contained 0.3 ml. of 3.7 per cent solution of acetylcholine (B.D.H., London) neutralized before use. This ACh was added to the flask solution about one hour after the filling of the flasks.

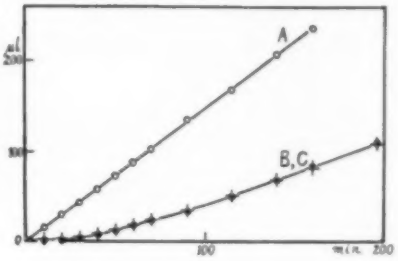

FIG. 10. Manometric determination of esterase activity. Enzyme preparation: Suspension of innervated portions of frog's muscles (170 mg. in each flask), at 15°C. Ordinates: CO_2—production in μl.; abscissae: time in minutes. *A*: normal Ringer; *B* (crosses): Ringer containing 10^{-5} eserine; *C* (full circles): Ringer containing 10^{-5} eserine +7.5 μmol. curarine.

It is clear from Fig. 10 that the addition of curarine (concentration 7.5 μmol. per l.), in no way influenced the rate of hydrolysis by the partially inhibited enzyme preparation (eserine concentration 10^{-5}).

Figure 10 shows that the activity of the eserinized enzyme solution increased gradually to a study rate after the substrate has been added, while the uninhibited enzyme acts at constant rate. Presumably this difference is due to a "competition" between inhibitor and substrate, *i.e.* to a gradual displacement of eserine from the eserine-enzyme compound by the added ACh, until a steady state is reached (13). Incidentally the results show that curarine in concentrations more than sufficient to block transmission does not inhibit the enzyme activity. With high concentrations (20 μmol. per l.) it was found to increase the eserine inhibition by about 25 per cent. This contrasts with a recent observation by Harris and Harris (27) who reported a marked inhibition of cholinesterase by a curare preparation "Intocostrin"; probably some substance other than curarine was responsible for this effect.

In two experiments intact muscles were soaked in 2 ml. of an ACh Ringer solution (1.5×10^{-5}) containing either eserine (1 to 2×10^{-5}) or eserine +curarine (9 μmol. per l.). After 3 to 4 hours at 18°C., the solutions were tested for ACh content by local application of a small quantity to a chronically denervated frog's sartorius which responded to ACh concentrations of less than 10^{-8}. There is no significant difference in the activities of the two solutions (*i.e.* less than a change of 2:1). It may be recalled that Dale, Feldberg and Vogt (12) found no appreciable change in the ACh

activity of perfusates of stimulated nerve-muscle preparations before and after curarization.

It can be concluded, therefore, that curarine does not antagonize the inhibition of cholinesterase by eserine.

2. Curare might curtail the endplate negativity by somehow quickening the rate of "accommodation" to the drug action. This was tested by recording the discharge of impulses in the frog's sartorius muscle following local application of ACh.

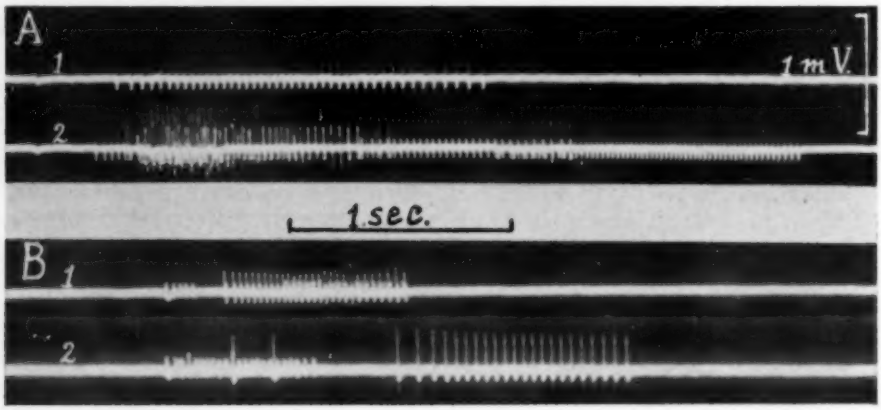

FIG. 11. Frog's sartorius. 16°C. Response to applied ACh. *A*. normal muscle. *1:* ACh 3×10^{-6}; *2*:ACh 10^{-5}. No discharges with ACh 10^{-6}. *B*. After 6 μmol. curarine. *1:* ACh 3×10^{-5}; *2:* ACh 10^{-4}. No discharges with ACh 10^{-5}.

The solutions were applied to the surface of the muscle by a platinum loop containing about 0.3 μ l. of solution and making contact with about 1 mm. of muscle length. The threshold concentrations of ACh varied from point to point, depending presumably upon the presence of superficial neuro-muscular junctions in close vicinity to the point of application. Invariably, however, the sensitivity to ACh was much lower at the nerve-free pelvic end than in the innervated parts. After a dose of curarine blocking neuromuscular transmission (3 to 6 μmol. per l.) the threshold concentrations for ACh rose 10 to 100 times, in contrast to those for KCl which were little, if at all, increased. For a given superthreshold concentration of ACh (*e.g.* 3 times that required to set up one or a few single fibre impulses, the duration of the repetitive discharge was sometimes a little shortened by curarization, Fig. 11; cf. Blair, 2). Delayed responses, and low-frequency discharges were still obtained. These observations show that, if there is any quickening of "accommodation" to ACh by curarine, it would be quite inadequate to account for the very drastic shortening of the eserine potential. In contrast with this, high-calcium Ringer (5 times the normal content), which is known to speed up accommodation to electrical and other stimuli, not only raised the threshold concentration, but reduced the discharge to one or two initial impulses and abolished any delayed response.

3. It might be that the prolonged eserine potential, particularly the slow wave, is not due to continuing ACh action, but is similar in nature to the prolonged negative after-potential such as is produced by veratrine. It is difficult to rule out this possibility entirely, but it must be remembered that the prolonged eserine-potential is restricted to the junctional region and that it is not produced by antidromic muscle impulses. Veratrine in low concentrations (2 to 5×10^{-8}) was found to enhance and prolong the negative after-potential of muscle (duration 2 sec. or more, size up to 20 mV) and cause repetitive responses, but it did not alter the size and time course of the e.p.p. due to an early second nerve volley.

It appears that some other explanation of the curare-eserine antagonism has to be sought. As outlined below all the observations of the present paper are compatible with the ACh hypothesis, if certain extensions of this hypothesis are made.

The facts on which the ACh hypothesis is based, are well-known: ACh is liberated by motor nerve impulses (12); it has a powerful and rapid stimulating action on the endplate region of the muscle (5, 6, 7); it is rapidly hydrolyzed by an enzyme concentrated at or near the endplates (39). The rapidity and chemical specificity of the ACh effect suggest that ACh produces its depolarizing action by combining with specific chemical receptors on the surface (9). Curarine, while apparently not affecting the ACh liberation, opposes its depolarizing action on the muscle membrane (10). Since, in addition to this specific blocking action, curarine chemically resembles ACh (both are quaternary ammonium cations, as also are other substances with similar actions, cf. Ing, 29), it seems likely that it also acts by combining with these same chemical receptors (cf. the receptive substance of Langley).

Eserine combines with the active centres of the cholinesterase molecules (13) and so blocks their hydrolyzing action on ACh. In addition eserine may have a weak chemical affinity to the muscle receptors, as is suggested by the fact that large doses of eserine diminish the endplate depolarization (Section I*B*).

If cholinesterase is highly concentrated at the endplate region (39), it would have a "barrier" effect preventing ACh from spreading to, and stimulating, adjacent muscle and nerve membranes. It has even been suggested (20, 38) that this may be the main or sole function of the esterase, and that the removal of ACh at the site of its release may be accomplished in a different way, *e.g.* by resynthesis to a chemical precursor.

In curarized muscle even in the presence of large doses of eserine the duration of e.p.p. and the underlying transmitter action is rather brief (Section I*B*), indicating that the removal of the transmitter still proceeds at a high rate. Some eserine-resistant mechanism of destruction must be present, whether it be due to a chemical restitution as suggested above, or to some cholinesterase accessible to ACh but not to eserine. Further in explaining the slow wave (cf. below) the additional assumption is made that this mechanism is strictly localized to the nerve-muscle junction, as indeed would be the case if it were a restitution process.

The "slow wave" set up in eserinized muscles (Section I*A*) indicates that there is a separate delayed action of the transmittter while its initial effect is declining. The relatively rapid spread of substances along interfaces to which they have chemical affinity (28) provides a possible explanation of this. ACh combining with membrane receptors would spread along the muscle surface from receptor to receptor and involve an increasing area of muscle membrane adjacent to the endplate. In the absence of eserine, this spread is prevented by the enzyme "barrier." In eserinized muscle, while ACh may still be inactivated at the endplate by a rapid, eserine-resistant mechanism (see above), some of it can spread beyond the range of this destruction and so give rise to the delayed slow wave. With a small dose of eserine, a single nerve volley is not sufficient to produce a slow wave. Summation of two or more nerve volleys is required (Section I*A*): the first few volleys help to maintain saturation of the remaining active enzyme molecules, while the successive quantities of ACh then can spread beyond the "barrier." Small doses of curarine greatly reduce the slow wave (Section I*B*). This might be attributed to the combination of curarine with the surface receptors (see above); curarine would diminish the rate of surface spread of ACh by blocking its combination with the receptors. Moreover, in this way curarine would

increase the chances of free ACh combining with active enzyme molecules and so enhance somewhat the rate of hydrolysis. Presumably this would account at least partly for the shortening of transmitter action by curarine in both eserinized and eserine-free muscle (Section I*B*; and 17).

Thus by making plausible assumptions, the observed curarine and eserine actions may readily be reconciled with the hypothesis that ACh, liberated by nerve impulses, is responsible for all the local potential changes at the neuromuscular junctions.

Summary

Both in cat's and frog's muscles eserine increases and lengthens the local negative potential change (the endplate potential, e.p.p.) set up by one or more nerve volleys at the myoneural junction.

In addition, repetitive nerve volleys produce a delayed "slow wave" (height up to 40 per cent of spike, duration up to several seconds) at the junctional region of eserinized muscle. The onset of the slow wave merges more or less with the decaying phase of the e.p.p.

Curare antagonizes the eserine effect: it greatly reduces the slow wave component, and shortens and diminishes the e.p.p. Even in fully curarized muscle, eserine causes a prolongation of the e.p.p.; the rising phase is lengthened up to threefold, the e.p.p. thus building up to a greater height. Both in normal and curarized muscle increase of eserine beyond a certain high concentration (about 3×10^{-5} in the frog) causes no further prolongation of the negative potentials, and actually diminishes their size.

The junctional potentials of eserinized muscle give the typical effects of an increased and prolonged catelectrotonus: (i) repetitive muscle spikes, when above threshold size; and (ii) lengthening of refractory period, and, during very intense depolarization, a complete block of impulse propagation.

The production of nerve after-discharge in the eserinized cat has been further investigated. Repetitive muscle impulses can arise from the junctional potentials independently of nerve after-discharge. In the cat, during prolonged eserine potentials, retrograde propagation of impulses from muscle to nerve was observed.

The principal effect of eserine is a lengthening of the action of the neuromuscular transmitter, thus leading to prolonged junctional negativity with consequent catelectrotonic effects.

In an attempted analysis of the curare-eserine antagonism it is shown that (i) curare does not antagonize the inhibition of cholinesterase by eserine; (ii) curare has little or no effect in quickening the adaptation of muscle to acetylcholine.

By making plausible assumptions it is shown that the observed curare and eserine actions are reconcilable with the hypothesis that ACh is responsible for all the local potential changes set up by nerve impulses.

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EXCITABILITY OF CEREBRAL CORTEX IN INFANT *MACACA MULATTA**

MARGARET A. KENNARD AND W. S. McCULLOCH
Laboratories of Physiology and Neurophysiology, Yale University School of Medicine, New Haven, Connecticut

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UNTIL THE second month, when voluntary movements begin, the motions of the infant monkey (*Macaca mulatta*) suggest that its cerebral cortex has little function. Some it must have, however, for cortical ablations cause some alterations of movement, and stimulation of area 4 at that age elicits some movements. Such responses to stimulation by induced currents from a Harvard coreless inductorium have already been reported from this Laboratory (3), and responses to 60-cycle stimulation of the infant cortex have been minutely investigated by Hines and Boynton (2) at various stages from fetal life to the end of the first year.

However, facilitation, extinction and suppression of motor response to cortical stimulation had never been studied in the infant monkey. The type and location of stimulation required to demonstrate them in the adult monkey are well known (1). Briefly, at any motor focus repetition of appropriate stimulation at short intervals yields facilitation; at longer intervals, extinction; and stimulation of any suppressor area (*e.g.* 4-s or 8) prevents response to subsequent stimulation of all motor foci. Those foci for specific discrete responses occupy (Brodmann's) area 4, and those for complex responses lie in area 6.

Since these characteristics have been demonstrated unequivocally in a single experiment upon a monkey 20 days old, the results appeared worth reporting. They are the more significant because the infant was normal for its age in weight, behavior and in the electroencephalogram. It exhibited normal righting reflexes, stood and walked on a wide base. It clung, climbed and sucked readily, and exhibited forceful and sustained reflex grasping as soon and as long as the palm or sole was touched. Its EEG before anesthesia showed typical low-voltage waves at 4–5 per sec.

PROCEDURE

A twenty-day old infant monkey (*Macaca mulatta*), wt. 515 gm., was fully anesthetized with 0.16 cc. Dial (Ciba), one-half intraperitoneally and one-half intramuscularly, and its cortex exposed for bipolar stimulation. Electrical pulses of controlled wave form, frequency and voltage were delivered by a stimulator having an output unaffected by the resistance between the electrodes. Motor responses were noted by three observers. Subsequently the cortex was locally strychninized and the induced electrical disturbance of the cortex recorded from bipolar leads, with a six-channel Grass electroencephalograph.

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Results

Areas yielding motor response. Bipolar electrical stimulation, even with impulses of abrupt rising phase and ten msec. falling phase, of relatively high voltage and of any frequency and duration, elicited motor responses only from the posterior margin of area 4—not from its anterior portion, nor from area 6, nor from the postcentral convolution. Suppression of these motor responses was produced, with the usual latency of several minutes, by stimulation of area 8 above the sulcus arcuatus (Fig. 1, pt. 51) and by

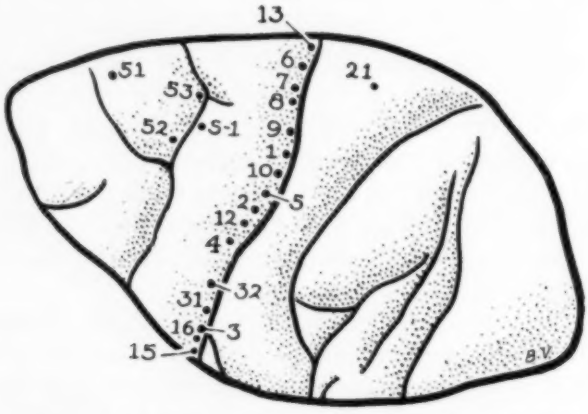

FIG. 1. (*Macaca mulatta*) operative exposure left hemisphere 20th day infant. For symbols, see text.

stimulation of area 4-s (pt. s-1). The locations and sequence of stimulations are given in Fig. 1, and the corresponding responses were as follows:

1. Shoulder, followed by whole arm
2. Thumb adduction, followed by flexion and supination at wrist
3. Tongue and upper lip
4. Thumb
5. At times extension and eversion of wrist, at times extension of thumb
6. No response usually, twice questionable response of shoulder
7. Thigh once, shoulder once
8. Change in respiratory rate, no abdominal contraction
9. Outward rotation of shoulder
10. Forearm and wrist
12. Thumb followed by 2nd and 3rd finger
13. Hamstrings, flexion of leg
15. Tongue and upper lip
16. Tongue and upper lip
21. No response
31. Lower jaw
32. Upper lip
52. No response
53. No response
S-1. Suppression
51. Suppression

Character of response. It should be noted that movements of the proximal portions of the extremities were elicited more easily and regularly than those of the distal parts; that responses of the face were well developed, those of the hand, thumb and finger, much less well defined and those of the foot and toes absent.

Compared with those obtained from the adult, the movements themselves were less constant, less discrete and slower in onset, execution and termination. Moreover, the threshold of motor foci was higher, no responses

could be elicited even with long, high voltage pulses at 1 per sec. and the responses to 5 pulses per sec. were not prompt and separate, as in the adult, but late and fused. This is obviously due to facilitation which was easily demonstrated by repetition of stimulation with short intervals. With intervals of about 15 seconds there was total extinction of motor response.

The threshold for suppression of motor response, both in areas 8 and 4-s, was like that of the adult, and the motor after-discharge provoked by stimulation in area 4 was held in abeyance by application of the same stimulation to area 4-s (Fig. 1. S-1)

Strychninization. Finally, local strychninization of area 4 produced typical strychnine spikes, and strychninization of area 4-s (at S-1) induced a typical suppression of electrical activity of the cortex.

Discussion

The responses to stimulation of the cortex of this three-week old macaque indicate that area 4 is already capable of initiating those slow and ill-differentiated movements which are typical of its age. Nor is it surprising that these are obtained from area 4 which is first to develop and first myelinated. What is of interest is that among the descending systems the shorter (to face and even to hand) are nearer to adult performance than the longer (to leg).

Since facilitation and extinction of motor response have both been demonstrated in the infant cortex, the interpretation of the slow and only relatively discrete motions deserves consideration. Since, as judged by motor response, the threshold is higher, the lack of separate responses to pulses at 1 per sec. and the tardy and fused responses to 5 per sec. may be taken to mean either that synchronous discharges leaving the cortex reach the cord late, scattered and decimated, or that the threshold of the cells of the cortex is itself higher. The latter seems unlikely, for 4-s and 8 have normal thresholds, though they are developmentally behind area 4. If the former is the fact, then one would have to expect less discrete response because of facilitation of neighboring motor foci and perhaps partial extinction of the focus under the electrode. In this case the apparent lack of differentiation of the cortical foci need not imply any inactivity of cortical elements,—merely a difference in the descending system. Be that as it may, the difference in the discreteness of motor response is not an artifact of stimulation, for the infant monkey in using its cortex would encounter the same difficulty. This is confirmed by the conformity of normal activity to that induced by cortical stimulation.

The failure to elicit motor responses from area 6 in the infant which still exhibits forced grasping is in harmony with the results of removal of area 6 in the adult, which brings on forced grasping.

That removal of 4-s at any age gives rise to spasticity only after the first six months of life, and that excitation of 4-s even in the third week gives suppression, suggest that the as yet unknown structure whose unsuppressed activity would yield spasticity is not yet active in the infant.

Summary

The cerebral cortex of the three-week old (infant) *Macaca mulatta*, when stimulated electrically, produces movement contralaterally.

The stimulable motor foci are confined to area 4 and no responses are elicitable from area 6 or the postcentral convolution.

Facilitation and extinction of motor response are easily demonstrable.

Relatively high voltages are required to elicit responses and these, like the spontaneous motions, are less discrete and slower in onset, execution and cessation than those of the adult. This may indicate either higher threshold in an as yet undeveloped cortex or less adequate corticospinal conduction.

Responses are more easily elicited in the face, less in the hand and not at all in the leg.

Lack of response from area 6 is correlated with the forceful, sustained and easily elicited forced grasping normal for that age.

That suppression of motor response is elicitable from 8 and 4-s at this age when removal of 4-s does not yet produce spasticity suggests the functional immaturity of that structure whose unsuppressed activity would give rise to spasticity in older animals.

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EFFECTS OF PRESYNAPTIC VOLLEYS ON SPREAD OF IMPULSES OVER THE SOMA OF THE MOTONEURON

BIRDSEY RENSHAW

From the Laboratories of The Rockefeller Institute for Medical Research, New York

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INTRODUCTION

RECENTLY the nature of impulse conduction at the soma (cell body and dendrites) of the neuron has been subjected to direct experimental examination (5, 6). The potential changes which may be recorded at a small needle electrode placed in a cranial motor nucleus reveal that stimulation of a motoneuron produces, in addition to the nerve impulse which sweeps over its axon, a response which must be attributed to the soma. This response is approximately the same whether the motoneuron be stimulated synaptically or antidromically. An impulse initiated in the axon or at some portion of the soma must therefore spread, at least to a certain extent, over the soma. Lorente de Nó has also shown that the electrical sign of activity at the soma is decreased in the successive responses to a rapid series of antidromic volleys. Asphyxia blocks passage of the antidromic impulses from the axons into the somas of the motoneurons.

The present experiments reveal that responses of the somas of spinal motoneurons resemble those of cranial motoneurons. It is also shown that the electrical response of the motoneuron soma may be augmented or, as the case may be, depressed by the central effects of sensory volleys. Even conditioning dorsal root volleys which fail to set up impulses in the axons of the tested motoneurons suffice to produce these alterations in the character of conduction at the soma.

METHODS

The experiments were carried out on rabbits and cats which had been either decerebrated or lightly narcotized with pentobaribtal sodium ("Nembutal"). After a laminectomy had been performed and the necessary spinal roots prepared, the cord was covered with a layer of paraffin oil. Conditioning afferent volleys were set up by the application of single shocks to groups of dorsal rootlets. Centripetal testing volleys were initiated in groups of motor axons either by stimulation of ventral roots which had been severed as they passed through the dura mater, or by stimulation of the central ends of cut peripheral motor nerves. In the latter case it was necessary to cut dorsal roots to prevent the entrance into the cord of impulses in sensory fibers of the stimulated nerve. Records were made of the potential differences which arose between a micro-electrode inserted into the ventral horn and a second, indifferently placed electrode. The micro-electrodes were steel needles of shank diameter 50 micra. They were insulated with enamel except at their tips, which had been ground at a gradual taper to sharp points. The usual differential amplifier, oscillograph and stimulating apparatus were used.

RESULTS

1. *Response of spinal motoneurons to antidromic stimulation.* The present experiments on motoneurons of the lumbo-sacral cord have confirmed several facts discovered by Lorente de Nó (5, 6) in his studies on the neurons of the

3rd, 6th and 12th cranial motor nuclei. In particular, (i) there is an electrical response of the soma which may be differentiated from that of the axon, and (ii) the response of the soma is much more subject to modification than the impulse (spike potential) in the axon. As shown by Gasser (1, p. 144), the shape of the axonal spike is remarkably constant, and under the conditions of the present experiments can not be expected to show any change.

The illustrative records of Fig. 1 have been taken from an experiment upon a decerebrated cat. After performing a laminectomy and opening the dura, the seventh lumbar (L_7) ventral root and the L_6 and L_7 dorsal roots were cut intradurally. The severed ventral root was raised on stimulating electrodes through which shocks maximal for alpha fibers were delivered. The centripetal motor volleys so initiated produced potential changes which were recorded at two loci.

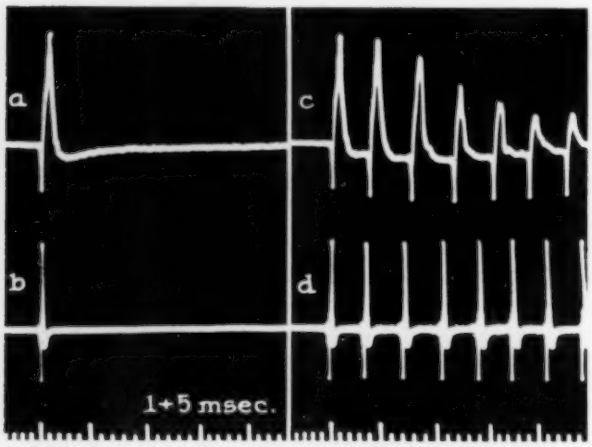

FIG. 1. Comparison of the responses of motoneuron somas (*a*, *c*) and of motor axons (*b*, *d*). Decerebrated cat. Records *a* and *c* were obtained with a micro-electrode in the ventral horn; *b* and *d*, with a small Ag-AgCl ball electrode on ventral root axons. In each case an electrode at a distant point completed the recording circuit. In this and the subsequent figures an upward deflection indicates negativity at the micro-electrode. The stimuli were single (*a*, *b*) and repetitive (*c*, *d*) shocks, maximal for alpha fibers, delivered to the ventral root. The amplification for records *a* and *c* was 5 times that for *b* and *d*.

(i) In the case of records *b* and *d* of Fig. 1 the active recording lead was a small Ag-AgCl ball electrode placed on the ventral root axons between their point of emergence from the cord at L_7 and the distally located stimulating electrodes. At this point the motor axons lay ventrolaterally beside the sacral cord; they were partially surrounded by a conducting medium consisting of the cord, the dura, a small amount of accumulated fluid and the underlying bone of the vertebral column. The rapid triphasic response (record *b*) is characteristic of the sequence of potential changes which are produced in a volume conductor at points close to a bundle of nerve fibers which conduct a nearly synchronous volley of impulses. The motor axons followed the stimulating shocks at high frequencies, for it is seen (record *d*) that successive centripetal motor volleys set up at a frequency of *circa* 286 per sec. were identical.

(ii) A small electrode was inserted into the L_7 ventral horn (records *a* and *c*). The recorded sequence of potential changes was very different from that produced by the conducted spike potentials in the motor axons. In

analogy with the findings of Lorente de Nó, record *a* from the ventral horn must be interpreted as follows. The initial downward deflection, which represented positivity at the micro-electrode, signalled the approach of the centripetal volley of impulses in the motor axons. The large negative (upward) deflection had a duration of somewhat more than 1 msec. It represented a depolarization of the somas of the motoneurons whose axons were occupied by the centripetal volley. Finally there appeared a much smaller and more prolonged positivity which indicated the existence of a difference in potential between the portions of the motoneuron somas near to the origin of the axons and the more terminal ramifications of the dendritic systems, the flow of current taking place in the direction from the cell bodies toward the dendritic tips.

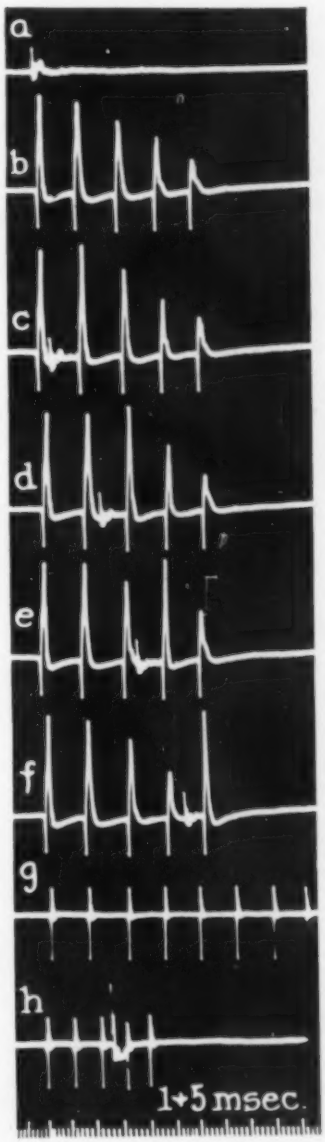

FIG. 2. Effect of dorsal root volley on response of motoneuron somas. Decerebrated cat. The recording microelectrode was in the ventral horn for records *a-f;* for records *g* and *h* it was inserted deep into the ventral white matter. *a*, L_7 dorsal root volley in isolation; *b*, 5 L_7 ventral root volleys in isolation; *c-f*, the dorsal root volley interpolated during the series of ventral root volleys; *g*, series of L_7 ventral root volleys; *h* interpolation of L_7 dorsal root volley in series of 5 L_7 ventral root volleys recorded from the ventral white matter.

The negative deflection, which signalled the entrance of the antidromic impulses into the somas of the motoneurons, did not maintain its full size in the successive responses to repetitive stimulation. In the case of record *c* of Fig. 1, for instance, the sixth response was reduced to one-third the height of the first. This alteration of the successive responses of the somas was not due to a comparable reduction in the size of the centripetal volleys in the motor axons for, as has been stated, these remained essentially unchanged (record *d*). The response of the soma of the motoneuron is therefore altered, and judging by the size of the negative deflection, is reduced at frequencies of stimulation which its axon is able to follow without difficulty.

2. *Conditioning by dorsal root volleys of the motoneuron response to antidromic stimulation.* The responses of motoneuron somas to stimulation by

centripetal impulses in their own axons are readily altered by the spinal activity due to arrival at the cord of impulses in sensory fibers (Fig. 2–4). In general the central effects produced by a dorsal root volley include both the changes due to activity in the primary afferent neurons themselves and the changes due to stimulation of intraspinal neurons. Depending on the tested motoneurons and on the population of sensory fibers occupied by the conditioning volley, the response of the motoneurons may be facilitated or inhibited; that is, the negativity referable to the invasion of the motoneuron soma by the testing centripetal motor impulses may be either augmented or reduced.

The records of Fig. 2, taken from the same experiment as those of Fig. 1, reveal a striking facilitatory effect of dorsal root volleys on motoneuron responses made subnormal as a result of repetitive antidromic stimulation. The active recording lead for records *a-f* was a micro-electrode inserted into the L_7 ventral horn. Record *a* shows the response to an L_7 dorsal root volley. In record *b* are seen the potential changes which arose as a result of the delivery of five shocks to the ventral root. There was a progressive decrease in the magnitude of the motoneuron response. In record *c* a dorsal root shock was applied during the interval between the initiation of the first and second motor nerve volleys. The responses to the second and third antidromic volleys were then much greater than in record *b*. It is noteworthy that the second response was not only greater than the second response when no dorsal root volley had preceded. It was also slightly but significantly larger than the response to an unconditioned antidromic stimulus, as may be seen by comparing the second response of record *c* with the first response of records *b-f*. In records *d* to *f* the dorsal root shock fell progressively later in the series of ventral root volleys. In each instance the subsequent motoneuron discharges were facilitated. Even the small response of the motoneurons to the fifth antidromic volley was facilitated by the preceding dorsal root volley (record *f*) to a size at least as large as the response of the motoneurons to the first (unconditioned) antidromic volley.

In contrast, the centripetal volleys in the motor axons were not altered in a comparable way. Records *g* and *h* of Fig. 2 were taken after the recording micro-electrode had been pushed deep into the ventral white matter, perhaps even into the bundles of motor axons which underlie the cord itself. The recorded responses therefore represent mainly the activity of motor axons. It is seen that, in contrast with the responses of the somas, the triphasic axon spike potentials remained constant during repetitive stimulation at even higher frequencies than were used in the case of oscillograms *b-f*. The only effect of an interpolated dorsal root volley was slightly to reduce, rather than to augment, the size of the subsequent antidromic volley (record *h*). The reduction can be explained by the refractoriness of some of the motor axons at the stimulating leads, consequent upon their having conducted reflex discharges, and by the collision of centrifugal motor reflex impulses with the centripetal testing impulses.

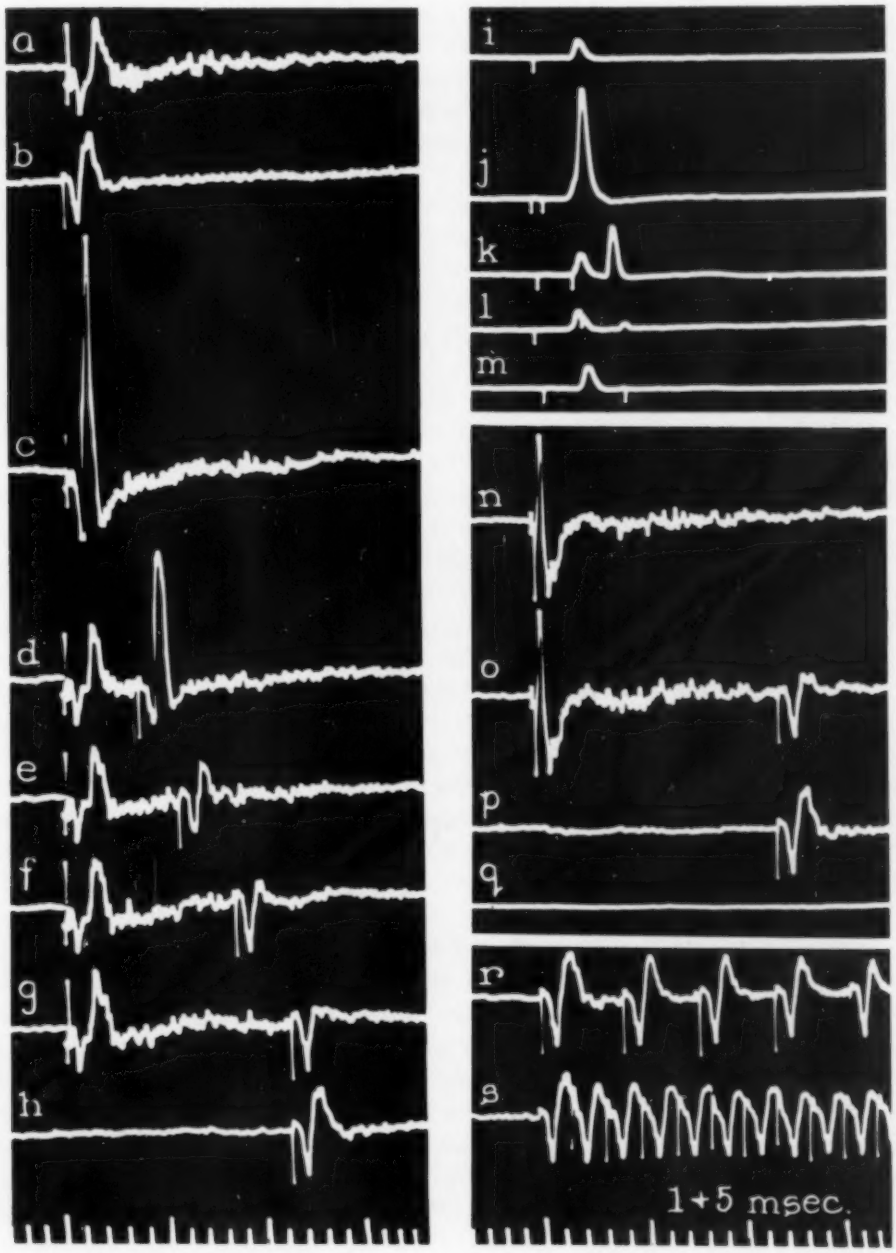

FIG. 3. Excitatory and inhibitory effects of dorsal root volleys on responses of motoneuron somas. Cat under light nembutal, dorsal roots cut. Oscillograms *a-h*, *n-p*, *r* and *s* are micro-electrode records from the L_6 ventral horn. *i-m* and *q* are records from the crural nerve. *a*, L_6 dorsal root volley. *b* and *h*, antidromic volley in crural motor axons. *c-g*, L_6 dorsal root volley conditioning crural antidromic volley. Records *i-m* show reflex discharges set up in crural motor axons by one and two L_6 dorsal root volleys. *n*, L_7 dorsal root volley. *p*, crural antidromic volley. *o*, L_7 dorsal root volley conditioning crural volley. *q* shows absence of conspicuous reflex discharge into crural axons following L_7 dorsal root volley. *r* and *s*, series of crural antidromic volleys.

The records of Fig. 3 illustrate both facilitatory and inhibitory actions of dorsal root volleys upon the somas of a pool of motoneurons. The preparation was a cat under Nembutal anesthesia. The caudal cord was transected and the ipsilateral dorsal roots cephalad through L_2 were cut. The tested motoneurons were those supplying the muscles of the quadriceps group. The testing centripetal motor volleys were initiated by the application of stimuli to the cut central ends of the nerve branches to the quadriceps. Conditioning volleys were set up in the L_6 and the L_7 dorsal roots. A micro-electrode was inserted into the lateral portion of the ventral horn at an axial level corresponding to the middle of the L_6 segment. This locus is within the pool of motoneurons which supply the muscles of the quadriceps group (10, 7).

The potential changes produced at the micro-electrode reveal that the somas of the tested (quadriceps) motoneurons were affected by the central effects produced by the arrival at the cord of the L_6 dorsal root volleys (records *a-h*). Record *a* shows the effect of an L_6 dorsal root volley in isolation; records *b* and *h* the responses to stimulation of the deafferented nerve branches to the quadriceps muscle. When the shock to the L_6 dorsal root and that to the quadriceps nerve were delivered in sequence so that the antidromic volley arrived at the cord shortly after the arrival of the impulses in the dorsal root fibers (record *c*, also *d*), the response of the motoneurons was greatly augmented. When, however, the antidromic volley arrived a few msec. after the dorsal root impulses, the motor impulses failed to enter the somas of the motoneurons to any extent. The inhibition of the response of the somas was nearly complete (records *f* and *g*). The time course of the conditioning of the response of the somas of the motoneurons to the antidromic volley roughly paralleled the conditioning by an L_6 dorsal root volley of the response of the motoneurons to a subsequent L_6 dorsal root volley (records *i-m;* cf. 9).

Careful comparison of records *c-g* with *i-m* of Fig. 3 reveals, however, that the response of the motoneuron somas to antidromic stimulation and the reflex discharges of motoneurons of the same pool were not conditioned in exactly the same time course. The conditioning sensory volley facilitated the direct response of the motoneuron somas to a centripetal motor volley for a longer period than it facilitated the reflex discharge to a dorsal root testing volley. The available data do not permit resolution of the alternative explanations for this difference.

In preparations such as the one under consideration an L_7 dorsal root volley typically fires no quadriceps motoneurons (Fig. 3 *q*) and has only an inhibitory effect on the reflex discharge of these motoneurons to a simultaneous or subsequent L_6 dorsal root volley (9). Correspondingly the response of the quadriceps motoneurons to a centripetal volley in their own axons, as shown in isolation in record *p*, was greatly depressed by a preceding L_7 dorsal root volley (record *o*). It is obvious that in this case the depression of the response of the somas could not have been due to subnormality of

motoneurons, since none were fired by the conditioning afferent volley.

It is apparent that the properties of motoneurons are altered by activity in the cord even when this activity itself does not fire the motoneurons. The degree to which the activity in sensory fibers and premotor neurons determines the state and excitability of the motoneurons is emphasized by noting that the motoneuron soma response deficit produced by an antecedent L_7 dorsal root volley which fired no motoneurons (record *o*) was greater than the deficit produced by direct repetitive activity of the motoneurons at high frequencies (records *r* and *s*).

The conspicuous augmentation of the response of the motoneuron somas has a delay, measured from the time of arrival of the dorsal root impulses, which is comparable with the synaptic delay at motoneurons as determined by Lorente de Nó (3, 4) and Renshaw (8). The illustrative records of Fig. 4 were taken from an experiment on a rabbit under light Nembutal anesthesia. The caudal cord was severed and all the dorsal roots on one side as far cephalad as L_2 were cut intradurally. Stimulating electrodes were placed on the tibial nerve in the thigh and on the combined ipsilateral dorsal rootlets of the L_7 and S_1 segments. Records were taken of the potential changes produced at a micro-electrode placed in the ventral horn. Record *a* shows the response to the dorsal root volley alone, and *b* the response to a tibial antidromic volley in isolation. In both records the time of arrival of the impulses at the ventral horn was approximated by the moment at which the initial positive deflection passed through the baseline to rise into the negative spike. Both the sensory impulses and the antidromic motor volleys were initiated in each of the subsequent oscillograms (*c-g*). Records *c* and *d* are approximately algebraic summations of the two responses in isolation.

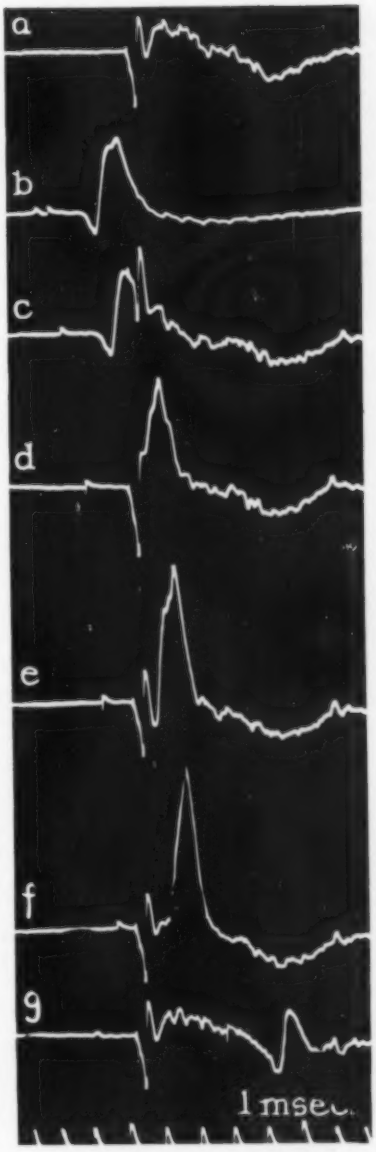

FIG. 4. Relation between time of arrival of dorsal root volley at the spinal cord and its conditioning effect on the somas of motoneurons. Rabbit under light nembutal, dorsal roots cut. Micro-electrode records from the ventral horn. *a*, L_7 plus S_1 dorsal root volley. *b*, tibial antidromic volley. *c-g*, both.

When, however, the dorsal root volley definitely preceded the arrival of the antidromic impulses (records *e* and *f*) it is seen that those portions of the motoneuron negativity which followed the arrival of the dorsal root impulses by 0.9 msec. and a little more were definitely augmented. This interval of 0.9 msec. represents an average value for the duration of synaptic delays at spinal motoneurons as well as for the central reflex times of two-neuron reflex arc discharges (8).

Examination of any of the figures, particularly of Fig. 4, reveals that the augmentation and depression of the electrical response relegated to the motoneuron somas occur *immediately* upon arrival of the antidromic testing volley at the cord. Therefore the part of the so-called motoneuron soma response which is conditioned by sensory impulses could not in reality be due to neurons of the ventral horn which might be excited after a synaptic delay by impulses in the recurrent collaterals of the testing motor axons.

Discussion

A plausible interpretation for the present findings is that impulses are conducted with a decrement at the soma of the motoneuron. The central changes initiated by dorsal root volleys would then augment, or as the case may be decrease, the extent to which antidromic impulses penetrate into the cell body and the dendrites. Consequently an antidromic impulse would travel less far, or further, into the soma of a motoneuron than when unconditioned by a dorsal root volley. This would suggest that, in contrast to conduction of impulses along the normal axon (2, 11), the "factor of safety" for conduction at the soma is not large. Indeed, any factor of safety which may exist can be abolished as a consequence of the central effects initiated by an antecedent dorsal root volley, even by a volley which produces no reflex discharges of the tested motoneurons.

To illustrate the meaning of this conclusion, suppose that a motoneuron is excited by the detonator action of impulses in fibers which terminate on some particular portion of the soma. Whether or not the activity would spread over the soma to excite the axon would depend upon the excitability and responsiveness of the intervening regions of the soma. The state of these intervening regions of the soma would depend upon the excitatory and inhibitory effects exerted by the instantaneous state of activity in the cord, as well as by the previous activity of the soma itself.

This conclusion is based on experiments with cats and rabbits which had been subjected to the following experimental manipulations: (i) they were either decerebrated or lightly anesthetized; (ii) they had undergone a laminectomy; (iii) in each case at least one ventral spinal root had been cut; (iv) a small micro-electrode was necessarily inserted into the ventral horn from which the records were taken. However, it does not seem probable that any of these conditions could alter the properties of the motoneurons in a radical manner.

SUMMARY

A centripetal volley of impulses which arrives at the spinal cord over ventral root fibers of cats and rabbits produces a series of potential changes in the ventral horn. A brief initial positive potential-change signals the approach of the impulses in ventral root axons. A negative deflection of a duration of 1–2 msec. follows. It is due to the invasion of the motoneuron soma (cell body and dendrites) by the centripetal impulses. The negativity passes over into a much more prolonged and smaller positive phase. A rapid series of essentially identical centripetal volleys evokes negative deflections of diminishing size. It is concluded that the response of the motoneuron soma is modified by frequencies of stimulation (*e.g.* 300 per sec.) which the motor axons follow readily. These observations on spinal motoneurons confirm Lorente de Nó's findings for cranial motoneurons.

Conditioning dorsal root volleys alter the motoneuron soma response to centripetal testing volleys in motor axons. The negative potential-change in the ventral horn due to the motoneuron somas is increased, or as the case may be, depressed. The result depends upon the conditioning dorsal root fibers, the tested motoneurons, and the interval between the arrival at the cord of the conditioning and the testing volleys.

It is concluded that retrograde conduction in the motoneurons occurs with a decrement. The degree of penetration into the cell body and dendrites can be augmented or decreased by the consequences of activity in sensory and premotor neurons.

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